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hereby certify that the annexed is a true copy of the Provisional specification in
connection with Application No. PP 6174 for a patent by THE UNIVERSITY OF
QUEENSLAND filed on 25 September 1998.



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A U S T R A L I A

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PROVISIONAL SPECIFICATION

for the invention entitled:

"A METHOD OF MODULATING PLANT PHYSIOLOGICAL
PROCESSES AND GENETIC SEQUENCES USEFUL FOR SAME - II"

The invention is described in the following statement:

- 1A -

A METHOD FOR MODULATING PLANT PHYSIOLOGICAL PROCESSES AND GENETIC SEQUENCES USEFUL FOR SAME-II

FIELD OF THE INVENTION

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The present invention relates generally to a method for modulating plant physiological processes such as but not limited to resistance to plant pathogens, senescence, cell growth and the shape of cells, tissues and organs. The method of the present invention is predicated in part on the manipulation of starch metabolism and/or cell expansion as a means for example, of inducing
10 resistance to plant pathogens and to modulate senescence or to alter cell growth or shape. In one particular embodiment, the present invention contemplates a method of modulating plant physiological processes by manipulating amylase production in plant cells. Another particular embodiment provides the manipulation of cell shape and/or cell expansion.

15 BACKGROUND OF THE INVENTION

Bibliographic details of the publications numerically referred to in this specification are collected at the end of the description.

20 Genetic engineering is now an integral part of strategies to develop varieties of plants with commercially useful traits. Transposons have played an important part in the genetic engineering of plants to provide *inter alia* tagged regions of plant genomes to facilitate the isolation of genes by recombinant DNA techniques as well as to identify important regions in plant genomes responsible for certain physiological processes.

25

The maize transposon *Activator* (*Ac*) and its derivative *Dissociation* (*Ds*) comprise one of the first transposon systems to be discovered (1,2) and was first used to clone genes by Fedoroff *et al* (3). The behaviour of *Ac* in maize has been studied extensively and excision occurs in both somatic and germline tissue. Studies have highlighted two important features of *Ac/Ds* for
30 tagging. First, the transposition frequency and second, the preference of *Ac/Ds* for transposition in linked sites.

The use of the *Ac/Ds* system has been hampered by the difficulty of data interpretation due, for example, to the high activity of *Ac* in certain plants and insertions at unlinked sites arising from multiple transpositions rather than by a single event from the T-DNA. This problem was addressed by Jones *et al* (4), Carroll *et al* (5) and others where a two component *Ac/Ds* system was developed. In this system, the *Ds* elements were made by replacing the *Ac* transposase gene with a marker gene thereby rendering it non-autonomous. T-DNA regions of binary vectors were constructed by Carroll *et al* (5) and Scofield *et al* (6) carrying either a *Ds* element or a stabilised Activator transposase gene (*sAc*). The *Ds* element contained a reporter gene (eg. *nos:BAR*) which was shown to be inactivated on crossing with plants carrying the *sAc* (5). This is referred to as transgene silencing. It has been shown that transgene silencing is a more general phenomenon in transgenic plants (7, 8, 9). Many different types of transgene silencing have now been reported in the literature and include: co-suppression of a transgene and a homologous endogenous plant gene (10), inactivation of ectopically located homologous transgenes in transgenic plants (7), the silencing of transgenes leading to resistance to virus infection (11) and inactivation of transgenes inserted in maize transposons in transgenic tomato (5).

Gene silencing undoubtedly reflects mechanisms of great importance in the understanding of plant gene regulation. Other important mechanisms include anti-methylation sequences (see Australian Patent Application filed on 4 June 1998 entitled "Expression Modulating Sequences") and negative regulatory sequences (see Australian Patent Application filed on 4 June 1998 entitled "Expression Modulating Sequences-II").

In work leading up to the present invention, the inventors identified yet a further regulatory mechanism involved in controlling plant physiological processes. The mechanism involves modulating starch metabolism and/or cell shape and/or expansion and this in turn influences such phenomena as disease resistance, senescence, cell growth and the shape of cells, tissues and organs.

- 3 -

SUMMARY OF THE INVENTION

Throughout this specification, unless the context requires otherwise, the word "comprise", or variations such as "comprises" or "comprising", will be understood to imply the inclusion of a
5 stated element or integer or group of elements or integers but not the exclusion of any other element or integer or group of elements or integers.

Sequence Identity Numbers (SEQ ID NOs.) for the nucleotide and amino acid sequences referred to in the specification are defined following the bibliography. A summary of SEQ ID NOs: is
10 given in Table 1.

One aspect of the present invention contemplates a method for controlling physiological processes in a plant said method comprising modulating starch metabolism in cells of said plant.

15 More particularly, the present invention is directed to a method of inducing a physiological response in a plant said method comprising enhancing or facilitating starch metabolism in cells of said plant after the initial development stage.

Another aspect of the present invention provides a method of inducing a physiological response
20 in a plant such as but not limited to inducing resistance to a plant pathogen, enhancing or delaying senescence, modifying cell growth or altering the shape of cells, tissues or organs, said method comprising modulating synthesis of an amylase or functional derivative thereof for a time and under conditions sufficient for starch metabolism to be facilitated or inhibited.

25 Still another aspect of the present invention relates to a transgenic plant or a genetically modified plant exhibiting one or more of the following properties:

- (i) a non-developmentally silenced amylase gene;
- (ii) an amylase gene capable of constitutive or inducible expression;
- 30 (iii) a mutation preventing silencing of an amylase gene;

- 4 -

- (iv) a nucleic acid molecule proximal to an amylase gene and which substantially prevents methylation of said amylase gene; and/or
- (v) decreased amylase gene expression.

5 Another aspect of the present invention contemplates a method for controlling physiological processes in a plant said method comprising modulating cell shape and/or expansion in said plant.

More particularly, the present invention is directed to a method of inducing a physiological response in a plant said method comprising enhancing or facilitating the manipulation of cell
10 shape and/or expansion in said plant.

Still another aspect of the present invention provides a method of inducing a physiological response in a plant such as but not limited to inducing resistance to a plant pathogen, enhancing or delaying senescence, modifying cell growth or altering the shape of cells, tissues or organs,
15 said method comprising modulating expression of the *Dem* gene.

Yet still another aspect of the present invention relates to a transgenic plant or a genetically modified plant exhibiting one or more of the following properties:

- 20 (i) a non-developmentally silenced *Dem* gene;
- (ii) a *Dem* gene capable of constitutive or inducible expression;
- (iii) a mutation preventing silencing of the *Dem* gene;
- (iv) a nucleic acid molecule proximal to the *Dem* gene and which substantially prevents methylation of said *Dem* gene or demethylates the *Dem* gene; and/or
- 25 (v) decreased *Dem* gene expression.

Another aspect of the present invention contemplates a method for controlling physiological processes in a plant said method comprising modulating C metabolism in cells of said plant.

- 5 -

More particularly, the present invention is directed to a method of inducing a physiological response in a plant said method comprising enhancing or facilitating C metabolism in cells of said plant.

5 Still another aspect of the present invention provides a method of inducing a physiological response in a plant such as but not limited to inducing resistance to a plant pathogen, enhancing or delaying senescence, modifying cell growth or altering the shape of cells, tissues or organs, said method comprising modulating expression of a putative patatin gene or a functional derivative thereof.

10

Yet still another aspect of the present invention relates to a transgenic plant or a genetically modified plant exhibiting one or more of the following properties:

- (i) a non-developmentally silenced putative patatin gene;
- 15 (ii) a putative patatin gene capable of constitutive or inducible expression;
- (iii) a mutation preventing silencing of a putative patatin gene;
- (iv) a nucleic acid molecule proximal to a putative patatin gene and which substantially prevents methylation of said putative patatin gene or demethylates said putative patatin gene; and/or
- 20 (v) decreased putative patatin gene expression.

TABLE 1
SUMMARY OF SEQ ID NOs.

	SEQ ID NO.	DESCRIPTION
5	1	Nucleotide sequence of tomato α -amylase gene promoter
	2	Nucleotide sequence of potato α -amylase gene promoter
	3	Nucleotide sequence of genomic DNA upstream of <i>Dem</i> gene followed by <i>Dem</i> cDNA coding sequence
	4	Nucleotide sequence of putative <i>Dem</i> promoter
	5	Nucleotide sequence upstream of <i>Ds</i> insertion (ie. upstream of the <i>nos:BAR</i> gene) in a putative patatin gene in tomato
10	6	Nucleotide sequence downstream of <i>Ds</i> insertion (ie. downstream of the <i>nos:BAR</i> gene) in a putative patatin gene in tomato
	7	Nucleotide sequence of portion of putative tomato homologue of potato patatin gene
	8	Nucleotide sequence of portion of potato patatin gene

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 is a diagrammatic representation showing T-DNA regions of binary vectors carrying a *Ds* element (SLJ1561) of the transposable gene (SLJ10512)[5]. The *Ds* element carries a
 5 *nos:BAR* gene and is inserted into a *nos:SPEC* excision marker. The transposon gene *sAc* is linked to a 2':*Gus* reporter gene.

Figure 2 is a diagrammatic representation showing an experimental strategy for generating tomato lines carrying transposed *Ds* elements (5). F1 plants heterozygous for both the *Ds* and
 10 *sAc* T-DNAs are test-crossed to produce TC₁ progeny. The TC₁ progeny are then screened for lines carrying a transposed *Ds* and a reactivated *nos:BAR* gene.

Figure 3 is a representation of a sequence comparison between the potato α -amylase promoter [SEQ ID NO:2] (14) and the tomato α -amylase promoter [SEQ ID NO:1]. The location of the
 15 UQ406 insertion is shown in bold.

Figure 4 is a diagrammatic representation showing the chromosomal region of the tomato α -amylase, *Dem* and γ genes. The α -amylase and γ coding sequences are shown as shaded boxes and the *Dem* gene as an open box on the chromosome. The region of homology to the potato
 20 α -amylase promoter and coding sequence are shown on the figure.

Figure 5 is a photographic representation showing tissue and *in situ* distribution of *Dem* mRNA.
 a, Northern blot analysis of *Dem* expression in light-grown seedlings (LS), dark-grown seedlings (DS), shoot apices (SA), mature leaves (ML), young fruit (YF), roots (R), stem (S) and callus
 25 (C). b-d, *in situ* hybridization with a *Dem* antisense probe. b, shoot apical meristem of a 4 week-old plant. c, dormant auxiliary meristem. d, root apex.

Figure 6 is a photographic representation showing somatic tagging of the *Dem* locus. a, leaf showing the somatic tagging of the *Dem* locus. Light coloured sectors on the adaxial side of the
 30 leaf represent independent insertions of *Ds* in *Dem*. The appearance of the abaxial side of the leaf is the same as wild-type. b, Scanning Electron Microscope (SEM) of a somatic sector

showing abnormal and wild-type epidermal cells. The SEM shows a wild-type sector in the lower right hand half of the figure, and a mutant sector in the upper left hand side. Note that the epidermal and hair cells are larger on the wild-type sector.

5 **Figure 7** is a representation showing that the *Dem* gene is required for palisade cell expansion in the leaf. Transverse sections of (a) variegated and (b) wild-type leaves. **p** and **s** indicate a palisade cell and spongy mesophyll cell layers, respectively. Light green parts are indicated by **lg**, and green parts by **g**. Light green sectors lacking palisade cells are mutated by *Ds* insertion in the *Dem* gene.

10

Figure 8 shows PCR on intact tissue of *dem* sectors. **M**, 1 kb ladder. **1-10**, unique *Ds* insertions in *Dem* detected by PCR. Intact leaf tissues (mutant somatic sectors) were used as template in the PCR. PCR with oligonucleotide primers facing out of *Ds* and in the *Dem* coding sequence amplified unique fragments from each mutant sector, thereby confirming that the sectors shown
15 in Figures 6 and 7 are indeed mutant *dem* sectors.

Figure 9 is a diagrammatic representation showing an improved transposon tagging strategy using *Dem* as excision marker. The *sAc* and *Ds* parent lines are represented by the upper left and right boxes, respectively. Because the stabilised *sAc* is linked to the frameshift *dem* allele in one
20 parent, somatic revertants occur at the frequency of about 1 out of 4 in the F1 progeny. Each somatic revertant represents an independent transposition event. Chr4, chromosome 4 of tomato.

Figure 10 is a representation of the nucleotide sequence [SEQ ID NO:3] of genomic DNA from
25 651 bp upstream of the *Ds* insertion in UQ406 to the beginning of the *Dem* coding sequence, followed by the *Dem* cDNA sequence from the ATG start site at base pair 4097. The target sequences of UQ406 and *Dem* ATG are underlined. The *Dem* cDNA sequence is shown in italics and is underlined. The putative *Dem* promoter is 709 bases long beginning at nucleotide 3388 and ending just prior to the ATG, i.e. at position 4096 [SEQ ID NO:4].

30

Figure 11 is a photographic representation showing the dominant lesion mimic phenotype of UQ406. The leaf tissue on the left is wild-type, on the right is UQ406. Young and old leaves are shown in the upper and lower portions of the figure, respectively. No symptoms have been observed on young differentiating tissue of UQ406.

5

Figure 12 is a diagrammatic representation of the genetic derivation of plants containing independent somatic *dem* alleles. Somatic revertants were generated by crossing plants heterozygous for the *dem*⁺⁷ mutant allele linked to transposase (sAc,GUS) and plants heterozygous for the *dem*^{Ds} mutant allele. Revertant seedlings were selfed and GUS⁺ individuals
10 were identified. From 150 somatic revertants, four independent lines were produced carrying hundreds of independent *dem* alleles.

Figure 13 is a photographic representation showing a multicellular palisade mutant allele of the *Dem* locus. At the single-cell embryo stage, the plant possessing the multicellular palisade sector
15 shown carried a transposase gene and was heterozygous for a mutant frameshift allele and a wild-type allele of the *Dem* locus. During development, however, mutant *dem* sectors were produced due to the insertion of a *Ds* element into the wild-type allele. Wild-type palisade tissue is essentially composed of single long columnar cells. Some mutant sectors (due to *Ds* insertion) totally lack palisade cells (refer to the figure), whereas other mutant sectors have multicellular
20 palisade tissue composed of small, non-columnar cells.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

In accordance with the present invention, transposon-mediated tagging of tomato plants was shown to result in the identification of mutants exhibiting altered physiological properties. In particular, the insertion of a transposon in close proximity to the α -amylase gene resulted in continued or modified expression of the α -amylase gene past the initial development stage of the plant. In wild-type plants, negative regulatory mechanisms which may include methylation result in the non-expression of the α -amylase gene. In accordance with the present invention, modified expression of the α -amylase gene, post or after initial developmental stage, results in physiological attributes such as altered senescence, altered resistance to pathogens, modification of the shape of plant cells, tissues and organs and altered cell growth characteristics. It is proposed, in accordance with the present invention, that the altered physiological phenotype is due to modified starch metabolism by the continued or modified expression of the α -amylase gene. In particular, increased or modified expression of the α -amylase gene or otherwise continued or altered expression of the α -amylase gene post initial development results in cell death, i.e. cell apoptosis, but also induces or promotes resistance to pathogens.

Accordingly, one aspect of the present invention contemplates a method for controlling physiological processes in a plant said method comprising modulating starch metabolism in cells of said plant.

More particularly, the present invention is directed to a method of inducing a physiological response in a plant said method comprising inhibiting or facilitating starch metabolism in cells of said plant after the initial developmental stage.

25

The present invention is exemplified herein with respect to the effects of starch metabolism in tomato plants. This is done, however, with the understanding that the present invention extends to the manipulation of starch metabolism in any plant such as flowering plants, crop plants, ornamental plants, vegetable plants, native Australian plants as well as Australian and non-Australian trees, shrubs and bushes.

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- 11 -

Physiological responses contemplated by the present invention include but are not limited to cell apoptosis, senescence, pathogen resistance, cell, tissue and organ shape and plant growth.

In a particularly preferred embodiment, starch metabolism is stimulated, promoted or otherwise enhanced or inhibited by manipulating levels of an amylase and this in turn may lead to *inter alia* senescence or apoptosis as well as resistance to pathogens. Reference to "amylase" includes any amylase associated with starch metabolism including α -amylase and β -amylase. This aspect of the present invention also includes mutant amylases. In addition, the manipulation of levels of amylase may be by modulating endogenous levels of a target plant's own amylase, or an exogenous amylase gene or antisense, co-suppression or ribozyme construct may be introduced into a plant. The exogenous amylase gene may be from another species or variety of plant or from the same species or variety or from the same plant. The present invention extends to recombinant amylases and derivative amylases including fusion molecules, hybrid molecules and amylases with altered substrate specifications and/or altered regulation.

According to another aspect of the present invention there is provided a method of inducing a physiological response in a plant such as but not limited to inducing resistance to a plant pathogen, enhancing or delaying senescence, modifying cell growth or altering the shape of cells, tissues or organs, said method comprising modulating synthesis of an amylase or functional derivative thereof for a time and under conditions sufficient for starch metabolism to be modified.

Preferably, the amylase is α -amylase.

The manipulation of amylase levels may be by manipulating the promoter for the amylase gene, inhibiting or promoting negative regulatory mechanisms such as described in an Australian Patent Application filed on 4 June 1998 entitled "Expression Modulating Sequences - II" or introducing anti-methylation sequences such as those described in an Australian Patent Application filed on 4 June 1998 entitled "Expression Modulating Sequences". Alternatively, an exogenous amylase gene may be introduced or an exogenous promoter designed to enhance expression of the endogenous amylase gene.

The present invention further extends to a transgenic plant or a genetically modified plant exhibiting one or more of the following characteristics:

- (i) a non-developmentally silenced amylase gene;
- 5 (ii) an amylase gene capable of constitutive or inducible expression;
- (iii) a mutation preventing silencing of an amylase gene;
- (iv) a nucleic acid molecule proximal to an amylase gene and which substantially prevents methylation of said amylase gene; and/or
- (v) decreased amylase gene expression.

10

The term "proximal" is used in its most general sense to include the position of the amylase gene near, close to or in the genetic vicinity of the nucleic acid molecule referred to in part (iv) above. More particularly, the term "proximal" is taken herein to mean that the amylase gene precedes, follows or is flanked by the nucleic acid molecule. Preferably, the amylase is within the nucleic acid molecule and, hence, is flanked by portions of the nucleic acid molecule. Generally, the
15 amylase gene is flanked by up to about 100 kb either side of the nucleic acid molecule, more preferably up to about 10 kb, even more preferably to about 4 kb either side of the nucleic acid molecule and even more preferably up to about 10 bp to about 1 kb.

20 Accordingly, another aspect of the present invention is directed to an isolated nucleic acid molecule comprising a sequence of nucleotides which stabilises, increases or enhances expression of an amylase gene inserted into, flanked by, adjacent to or otherwise proximal to the said nucleic acid molecule.

25 In an alternative embodiment, the present invention contemplates an isolated nucleic acid molecule comprising a sequence of nucleotides which inhibits, decreases or otherwise reduces expression of an amylase gene inserted into, flanked by, adjacent to or otherwise proximal to the said nucleic acid molecule.

30

The term "expression" is conveniently determined in terms of desired phenotype. Accordingly, the expression of a nucleotide sequence may be determined by a measurable phenotypic change such as resistance to a plant pathogen, enhanced or delayed senescence, altered cell growth or altered cell, tissue or organ shape.

5

The nucleic acid molecule described above is referred to herein as an "expression modulating sequence" (EMS) since it functions to and is capable of modulating expression of an amylase gene or its derivatives. The term "modulating" includes increasing or stabilising expression of the amylase gene or decreasing or inhibiting the amylase gene. An EMS may be a co-suppression
10 molecule, ribozyme, antisense molecule, an anti-methylation sequence, a methylation-inducing sequence and/or a negative regulatory sequence, amongst other molecules.

Accordingly, another aspect of the present invention relates to an expression modulating sequence (EMS) comprising a sequence of nucleotides which increases, enhances or stabilizes
15 expression of an amylase gene inserted within, adjacent to or otherwise proximal with said EMS.

In an alternative embodiment, the present invention provides an expression modulating sequence (EMS) comprising a sequence of nucleotides which inhibits, decreases or otherwise reduces expression of an amylase gene inserted within, adjacent to or otherwise proximal with said EMS.
20

Another aspect of the present invention contemplates a genetic construct comprising an EMS as herein defined and means to facilitate insertion of a nucleotide sequence within, adjacent to or otherwise proximal with said EMS wherein said nucleotide sequence encodes an amylase or functional derivative thereof.

25

The term "genetic construct" is used in its broadest sense to include any recombinant nucleic acid molecule and includes a vector, binary vector, recombinant virus and gene construct.

The means to facilitate insertion of a nucleotide sequence include but are not limited to one or
30 more restriction endonuclease sites, homologous recombination, transposon insertion, random insertion and primer and site-directed insertion mutagenesis. Preferably, however, the means is

one or more restriction endonuclease sites. In the case of the latter, the nucleic acid molecule is cleaved and another nucleotide sequence ligated into the cleaved nucleic acid molecule.

Preferably, the amylase gene sequence is operably linked to a promoter in the genetic construct.

5

According to this embodiment, there is provided a genetic construct comprising an EMS as herein defined and means to facilitate insertion of a nucleotide sequence within, adjacent to or otherwise proximal with said EMS and operably linked to a promoter wherein said nucleotide sequence encodes an amylase or functional derivative thereof.

10

Conveniently, the genetic construct may be a transposable element such as but not limited to a modified form of *Ds*. A modified form of *Ds* includes a *Ds* molecule comprising an EMS and a nucleotide sequence such as but not limited to a reporter gene and a gene encoding an amylase.

15 Another aspect of the present invention contemplates a method of increasing or stabilising expression of a nucleotide sequence encoding an amylase or otherwise preventing or reducing silencing of a nucleotide sequence encoding an amylase in a plant cell said method comprising introducing into said plant or plant cells said nucleotide sequence encoding an amylase flanked by, adjacent to or otherwise proximal with an EMS.

20

In an alternative embodiment, the present invention provides a method of inhibiting, decreasing or otherwise reducing expression of a nucleotide sequence encoding an amylase in a plant cell said method comprising introducing into said plant or plant cells said nucleotide sequence encoding an amylase flanked by, adjacent to or otherwise proximal with an EMS.

25

Yet another aspect of the present invention provides a transgenic plant carrying a nucleotide sequence encoding an amylase flanked by, adjacent to or otherwise proximal with an EMS.

Still a further aspect of the present invention provides nucleic acid molecules encoding apoptotic peptides, polypeptides or proteins or nucleic acid molecules which themselves confer apoptosis.
30 One example of an apoptotic nucleic acid molecule is a molecule capable of inducing or

- 15 -

enhancing amylase synthesis. Other molecules are readily identified, for example, by a differential assay. In this example, nucleic acid sequences (e.g. DNA, cDNA, mRNA) are isolated from wild type plants and mutant plants which exhibit enhanced or modified amylase gene expression. The differential assay seeks to identify DNA or mRNA molecules in the mutant
5 plant or wild type plant which are absent in the respective wild type plant or mutant plant. Such nucleic acid molecules are deemed putative apoptosis-inducing or apoptosis-inhibiting genetic sequences. These molecules may have utility in regulating beneficial physiological processes in plants.

10 The present invention is further directed to the putative *Dem* promoter and its further derivatives. This is approximately 709 bases in length extending upstream from the ATG start site. The nucleotide positions of putative *Dem* promoter are nucleotide 3388 to 4096 (Figure 10).

Another aspect of the present invention contemplates a method for controlling physiological
15 processes in a plant said method comprising modulating cell shape and/or expansion in said plant.

More particularly, the present invention is directed to a method of inducing a physiological response in a plant said method comprising enhancing or facilitating the manipulation of cell shape and/or expansion in said plant.

20

This aspect of the present invention is based on the detection of a *Ds* insertion in the *Dem* gene in plants. The resulting mutation results in genetically-modified palisade tissue. Mutant lines exhibiting altered cell shape or expansion are selected and, in turn, further lines exhibiting such beneficial characteristics as increased levels of photosynthetic activity are obtainable.

25

Accordingly, another aspect of the present invention provides a method of inducing a physiological response in a plant such as but not limited to inducing resistance to a plant pathogen, enhancing or delaying senescence, modifying cell growth or altering the shape of cells, tissues or organs, said method comprising modulating expression of the *Dem* gene.

30

Still yet another aspect of the present invention relates to a transgenic plant or a genetically modified plant exhibiting one or more of the following properties:

- (i) a non-developmentally silenced *Dem* gene;
- 5 (ii) a *Dem* gene capable of constitutive or inducible expression;
- (iii) a mutation preventing silencing of the *Dem* gene;
- (iv) a nucleic acid molecule proximal to the *Dem* gene and which substantially prevents methylation of said *Dem* gene or demethylates the *Dem* gene; and/or
- (v) decreased *Dem* gene expression.

10

Yet another aspect of the present invention is directed to a mutation in or altered expression of a putative patatin gene in tomato or other plants. The patatin gene is referred to herein as "putative" as it exhibits homology to the potato patatin gene.

- 15 Accordingly, another aspect of the present invention contemplates a method for controlling physiological processes in a plant said method comprising modulating C metabolism in cells of said plant.

More particularly, the present invention is directed to a method of inducing a physiological
20 response in a plant said method comprising enhancing or facilitating C metabolism in cells of said plant.

Another aspect of the present invention provides a method of inducing a physiological response in a plant such as but not limited to inducing resistance to a plant pathogen, enhancing or
25 delaying senescence, modifying cell growth or altering the shape of cells, tissues or organs, said method comprising modulating expression of a putative patatin gene or a functional derivative thereof.

- 17 -

Still yet another aspect of the present invention relates to a transgenic plant or a genetically modified plant exhibiting one or more of the following properties:

- (i) a non-developmentally silenced putative patatin gene;
- 5 (ii) a putative patatin gene capable of constitutive or inducible expression;
- (iii) a mutation preventing silencing of a putative patatin gene;
- (iv) a nucleic acid molecule proximal to a putative patatin gene and which substantially prevents methylation of said putative patatin gene or demethylates said putative patatin gene; and/or
- 10 (v) decreased putative patatin gene expression.

The present invention is further described by the following non-limiting Examples.

EXAMPLE 1

Ds Transposon tagging of an α -amylase gene affecting plant development

The inventors have previously developed a two component *Ds/sAc* transposon system in transgenic tomato for tagging and cloning important genes from plants (5, 12). The components of the system are shown in Figure 1 and comprise: i) a non-autonomous genetically-engineered *Ds* element (e.g. SLJ1561), and ii) an unlinked transposase gene *sAc* (SLJ10512), required for transposition of the *Ds* element. To activate transposition, the two components are combined by crossing transformants for each component. A plant selectable marker gene, e.g. *nos:BAR*, is inserted into the *Ds* element to enable selection for reinsertion of the elements following excision from the T-DNA (Figure 1). Surprisingly, the marker gene is irreversibly inactivated when the *Ds* line is crossed to a transformant expressing the transposase gene (5). Silencing occurred when the *Ds* element remained in the T-DNA, and also occurred in the great majority of cases when the *Ds* element transposed to a new location in the tomato genome. None of the other marker genes in the T-DNA is silenced. The silenced marker gene has been shown to be stably inherited, even after the transposase gene segregates away from the *Ds* element in subsequent generations.

The experimental strategy for generating tomato lines carrying transposed *Ds* elements from T-DNA 1561E is shown in Figure 2. One line, called UQ406, carries a single transposed *Ds* element (without the transposase gene which has segregated away) and is characterised by showing a disease mimic or premature senescence phenotype on mature leaves. UQ406 also possesses an active *nos:BAR* gene indicating that the insertion caused two phenotypes; namely premature senescence and reactivation of the *nos:BAR* gene inside the *Ds* element.

25

GenomeWalker (13) is used to clone the tomato DNA sequences flanking the *Ds* element in UQ406. The DNA flanking the *Ds* element in line UQ406 is cloned and sequenced, and a search of the PROSITE database reveals that the *Ds* has inserted into the promoter region of an α -amylase gene. The promoter shows strong homology to an α -amylase promoter of potato (14; Figure 3) and the coding sequence of the gene has strong homology with one of 3 reported potato α -amylase cDNAs (15). Surprisingly, DNA sequence analysis also shows that the *Ds*

- 19 -

insertion in UQ406 is located only about 3 kb upstream from the ATG of the *Dem* (Defective embryo and meristems) gene which has been cloned by tagging with *Ds*. In fact, only about 700 bp of DNA separates the putative α -amylase STOP codon and the *Dem* ATG codon (Figure 4). The *Dem* gene is required for correct patterning in all of the major sites of differentiation, namely in the embryo, meristems, and organ primordia (Figure 5). The inventors have shown by somatically tagging *Dem* with *Ds*, that the gene is involved in cell expansion during plant differentiation (Figures 6, 7 and 8). The close proximity of the α -amylase and *Dem* genes indicates that the α -amylase gene may also be involved in cell expansion during plant differentiation. The sequence flanking the active *nos:BAR* genes are referred to herein as

10 "Expression Modulating Sequences" or "EMSs".

EXAMPLE 2

An improved transposon tagging strategy for transgenic tomato

15 The inventors have used the transposon tagging system described in Example 1 (also see Figure 2) to tag and clone three important genes involved in shoot morphogenesis: the *DCL* gene, required for chloroplast development and palisade cell morphogenesis (12); the *Dem* gene, required for cotyledon development and shoot meristem function; and the α -amylase gene, described in Example 1 above.

20

Stable *Ds* insertion mutants of *Dem* germinate but fail to develop any further. However, variegated seedlings appear at first to be mutant, but the transposase gene activates transposition of the *Ds* and reversion of the *Dem* locus to wild-type, thereby restoring function to the shoot meristem. While the transposon tagging system described in Figure 2 has been successful in

25 tagging genes and chromosomal regions alleviating transgene silencing, it does have two associated inefficiencies. First, transposition cannot be selected in the shoot meristem of F_1 plants heterozygous for *Ds* and *sAc*. As a consequence, many TC_1 progeny derived from test-crossing these F_1 plants still have the *Ds* located in the T-DNA. The other limitation of the system is that sibling TC_1 progeny derived from a single F_1 plant often carry the same clonal

30 transposition and reinsertion event. The extent of clonal events amongst sibling TC_1 progeny can only be monitored by time consuming and expensive Southern hybridization.

These two inefficiencies in the transposon tagging strategy are overcome in accordance with the present invention by using the *Dem* gene as an excision marker. The new system enables selection for transposition in the shoot apical meristem and visual identification of plants carrying independent transposition events. Transposition is initiated by crossing a *Ds* line with a *sAc* line (Figure 9). The *Ds* line is heterozygous for a *Ds* insertion in the *Dem* gene and the *sAc* line is heterozygous for a stable frameshift mutation in the *Dem* gene (Figure 9). The frameshift allele is derived from a *Ds* excision event from the *Dem* locus. Both the *Ds* and *sAc* lines are wild-type due to the recessive nature of the *Ds* insertion and frameshift alleles. PCR tests on intact leaf tissue have been developed for the rapid identification of these *Ds* and *sAc* parental lines. The F_1 progeny derived from crossing the *Ds* and *sAc* lines segregate at the expected ratio of 3 wild-types to 1 mutant. Because the stabilised *sAc* is linked to the frameshift *dem* allele almost all of the F_1 mutants also inherit the transposase gene (*sAc*) and can undergo somatic reversion. These revertant individuals have abnormal cotyledons, but *Ds* excision from the *Dem* gene restores function to the shoot apical meristem. Each somatic revertant represents an independent transposition event from the *Dem* locus. A non-destructive test for *nos:BAR* expression is used involving application of PPT (the selective agent for expression of *BAR* gene) to a small area of a leaf. Somatic revertants resistant to PPT are grown through to seed and the F_2 progeny are screened again for PPT resistance. Lines carrying transposed *Ds* elements are selected for more detailed molecular analysis. Independent *Ds* insertions in the vicinity of *Dem* and the α -amylase gene are identified by PCR.

EXAMPLE 3

Modification of plant cell, tissues and organ shapes and plant growth by genetic manipulation of α -amylase

The DNA from 651 bp of the upstream of the UQ406 insertion down to the end of the *Dem* coding sequence has been sequenced (Figure 10). The close proximity of the α -amylase gene to the *Dem* cell expansion gene indicates that these genes may play a key role in cell expansion and differentiation. Several heterozygous insertion mutants are identified in the α -amylase coding sequence and these are selfed to produce plants homozygous for the *Ds* insertion in the α -

- 21 -

amylase coding sequence. If these have a similar or more or less severe phenotype to the plants homozygous for the stable *Dem* insertion mutant, then this will indicate that indeed this cloned α -amylase gene plays a key role in cell expansion, and, therefore, the shape and growth of plants. Several heterozygous insertion mutants have been identified in the γ coding sequence downstream of the *Dem* coding sequence (Figure 4) and these are selfed to produce plants homozygous for the *Ds* insertion in the γ coding sequence. If these have a similar or more or less severe phenotype to the plants homozygous for the stable *Dem* insertion mutant, then this will indicate that the γ gene also has a role in cell expansion and the shape and growth of plants.

10 A tomato chromosomal region spanning these genes is cloned into an *Agrobacterium* binary vector (16) to produce plasmid pUQ113, and this plasmid is introduced into *Arabidopsis* by method of (17) to modify the cell shape and growth of this other plant species. A T-DNA insertion mutant in the *Dem* gene is identified in *Arabidopsis* and this mutant is also transformed with pUQ113 to modify the cell shape and growth of *Arabidopsis*.

15

Recombinant combinations of α -amylase and *Dem* genes are transformed into a range of plant species to modify the cell shape and growth of the species.

EXAMPLE 4

20 Genetic engineering of disease resistance and senescence based on modification of expression of α -amylase

Ds insertion mutant UQ406 is characterized by a lesion mimic phenotype. The mutant phenotype is evident in mature leaves (Figure 11), but not in young leaves or any other tissue. No pathogens are found in leaf tissue displaying this phenotype. The dominant nature of the UQ406 phenotype and the location of the *Ds* in the α -amylase promoter suggest that over-, under or constitutive expression of the gene may be responsible for activating a disease resistance response and/or senescence in mature leaves. These data and the very close proximity of the α -amylase and *Dem* genes are also consistent with co-ordinate regulation of these genes in differentiating tissue.

30 Induction of disease resistance and plant senescence, to produce desirable outcomes in crops and

plant products, may, therefore, be able to be controlled by modification of α -amylase expression.

An early event in the disease response of a challenged plant is a major respiratory burst, often referred to as an oxidative burst due to an increase in oxygen consumption. This burst of oxygen consumption is due to the production of hydrogen peroxide (H_2O_2) linked to a surge in hexose monophosphate shunt activity (19). This activity results from the activation of a membrane-bound NADPH oxidase system which catalyses the single electron reduction of oxygen to form superoxide (HO_2/O_2^-), using NADPH as the reductant (19). Spontaneous dismutation of HO_2/O_2^- then yields H_2O_2 . Consumption of glucose *via* the hexose monophosphate shunt (alternatively known as the cytosolic oxidative pentose phosphate pathway) regenerates the NADPH consumed by the NADPH oxidase system. It is, therefore, entirely conceivable that an α -amylase is responsible for supplying sugars required by the pentose phosphate pathway, and perhaps for the primary activation of the signal transduction pathway that leads to disease resistance in plants.

15

Following the oxidative burst, disease resistance is manifested in localised plant cell death called the hypersensitive response (HR), in the vicinity of the pathogen. The HR may then induce a form of long-lasting, broad spectrum, systemic and commercially important resistance known as systemic acquired resistance (SAR). The compounds, salicylic acid, jasmonic acid and their methyl derivatives as well as a group of proteins known as pathogenesis related (PR) proteins are used as indicators of the induction of SAR (18).

20

Increased levels of sugars have been related to heightened resistance especially to biotrophic pathogens (20). When invertase (the enzyme responsible for the breakdown of sucrose to glucose and fructose) is overexpressed in transgenic tobacco, systemic acquired resistance is induced (21).

25

The α -amylase coding sequence is inserted behind an inducible promoter and transformed into plants to confer a inducible disease resistance in plants. Similarly, the α -amylase coding sequence is inserted behind an inducible promoter and transformed into plants to confer inducible senescence in plants for the production of desirable products or traits.

30

When a disease resistance response is invoked in one part of a plant, a general and systemic acquired enhancement in disease resistance is conferred on all tissues of such a plant (18). Tomato line UQ406 is tested for enhanced resistance to a wide range of pathogens to test this hypothesis.

5

EXAMPLE 5

Modification of the photosynthetic architecture of plants

A genotype has been produced for the somatic tagging of the *Dem* gene, thereby demonstrating
10 the involvement of the *Dem* gene in cell expansion. The genetic derivation of somatically-tagged *Dem* is shown in Figure 12.

Besides palisade-less sectors, another type of mutant sector has been identified in somatically-tagged *Dem* plants. The new phenotypic class is characterized by multicellular palisade tissue.
15 In the wild-type tomato, the palisade tissue is composed of a single long columnar palisade cell. In the new mutant sectors, which look wild-type to the naked eye, the long columnar cell is replaced by several smaller cells packed on top of one another. This is shown in Figure 13. Each mutant sector arises from an independent insertion of *Ds* in the *Dem* gene. The different classes of mutant sectors apparently result from different classes of mutations in the *Dem* gene.

20

Somatically-tagged *Dem* plants are crossed to a stable null mutant of *Dem* and progeny are screened to identify stable mutant lines with genetically-modified palisade tissue. Lines exhibiting beneficial characteristics, such as increased levels of photosynthetic activity, can then be selected. Lines resulting from other *Dem* alleles and exhibiting other beneficial modifications, for example
25 altered developmental architecture such as modified cell, tissue or organ growth rate, shape or form, may also be identified.

EXAMPLE 6

Ds transposon tagging of a putative patatin gene

Other lines carrying transposed *Ds* elements have also been selected.

5

DNA sequences flanking the active *nos:BAR* in a line designated UQ12 have similarly been cloned and sequenced. The flanking DNA appears to correspond to an intron in a homologous potato patatin gene. Patatin is the major protein in the potato tuber and has many potentially-important characteristics. For example, it possesses antioxidant activity; it has esterase activity
10 and is potentially a phospholipase or lipid acyl hydrolase (hydrolyzing phospholipids, liberating free fatty acids); it is induced during disease resistance; and it inhibits insect larval growth.

The sequence upstream of the *Ds* insertion (i.e. upstream of the *nos:BAR* gene) is as follows:

15	AATCAAAGAG GAATTNAATT CCNCAAAATT TCATCCATAG ATTTTGNGTC	50
	TCTGAAAATT AAAGTGACTT TGTAATCTGA AACCTAGAGT CCTCAACCAT	100
	ATCATTGACC ATTAAGCCAT ACCCTTAAAT GTAGGGAATT TGAAGTTTTA	150
	AAAACCACAC TTTGTTATTT ATTGGCCCAA ATACTCGATA ATCTTTACAT	200
	TATTGAAAAT CAACATTCAA AAGGAACGAA CCTTCAATCA CACCATCAAT	250
20	GTCAACTTTC TTTTATTTTG GATAATCTAA GTTTTAAAT TGCAGTAAAA	300
	TNAAATAAAA CCCTAAACTT CTTCTAGGTT GAGACTTAGT AAATATGAAT	350
	TATATAAAGA ATTCATGACA AATGAGACAT AAGAATAGTG CCAGCAAATT	400
	ACTTTTTTGA TATCTTATCT GTGATATCGG AATTTTAACT ACCATAAATT	450
	TATGAATGAA ATATCACTTA TCTATTAGAG AGGATTTAAT CTCCCTTATA	500
25	ATGACATTGA TAAAAGCAAG NACAAGTGCT CTTTATTTCT TAATTACAAA	550
	TCCTTAAATA GATAAAAGCT ACGAATAACA TAATATCCTT AAATAGATAA	600
	AAGCTACGAA TAACATAATA GTATATTACT CCNAATTATT TTGATTTATT	650
	TAAAATGACT CCACTAATCC TGATGTGGTC TAGG [SEQ ID NO:5]	684

30

5	GGTCTAGGCC	CTGGGCTCTAG	GAAACAAAAAT	AACCTATTGTG	ACTCCTAAAC	50
	AATAGCAACA	TACAAACCAC	TGATATTGTA	CAAGTAAAT	TCAATAAAAT	100
	TCTAGCTCTC	TCAAACACTT	TTAAAATTGT	TATTTCTGTT	TTGTCTGTGT	150
	CATATTATGA	CCTACACAAC	AACAACAACA	ACGAATTTAG	TGAAACTCTA	200
	CAAAGTGGAG	CCTGAAGTCG	AGAGTTTACG	CGGGCCTTAT	CACTATCTTT	250
10	TCGAGATAAA	AAAATTATTT	TTAAAAGATC	ATCGACTTAA	ACAAACCAAA	300
	CAATAATTAA	AAAAATATGA	ATTAATAGCA	AAGCAGTGTG	GACCATATAT	350
	ACAAAAATCT	ATAACAACAA	CAAGGTGCAG	AGCATTATTC	CAACTAAGAT	400
	CGAAGTTGTG	ATACTGTCTAT	AATAAAAAATG	ACACATATTT	TGACAACATA	450
	AAAAATAAAT	AACCATAAAA	TATATCATAG	AAAAATGAAT	ATATTAGAAC	500
15	AGCTCACTCC	AATATTAAAA	GAGAGAAAAA	AAATATTTTC	CCACCACAAT	550
	GCCATAATCC	TTGAGCTTAG	CTATTTATAA	GTAAAAAAA	TGTTTTCTTG	600
	GATAAATAGA	AAAAGAAATA	ATAATTAAAC	ATAACCAATC	ACTTCACAAA	650
	TAAGAGTGTA	TT	[SEQ ID NO:6]			662

```

20 Tomato: 307 ATTTATTTTTAGGAAAAATTATCTAAATACACATCTTATTTTACCATATACTCTAAAAAT 248
          | ||| ||||||||||||| ||||||||| ||| | || ||||| ||||| |
Potato: 1914 AATTATATTTAGGAAAAATTACATAAATACACAACCTTAATATATTATATTCTCTAAAATT 1973

          247 TCC 245 [SEQ ID NO:7]
25          |||
          1974 TCC 1976 [SEQ ID NO:8]

```

EXAMPLE 7

Tagging of additional genes involved in carbon metabolism

This *Ds* line also exhibits a disease mimic phenotype, indicating that the patatin gene may be involved in disease resistance and/or may act as an anti-oxidant in plant cells.

Selecting for transposition of a methylated *Ds* from the *Dem* locus and for expression of the *nos:BAR* gene (i.e.: demethylation of the *Ds*) efficiently identifies *Ds* insertions into genes, as opposed to so-called "junk DNA". The sequences adjacent to five of these *Ds* insertions have been cloned and sequenced, and in all of the cases the *Ds* insertion is in the vicinity of a known gene.

The five lines carrying active *nos:BAR* genes associated with genes are:

- *Ds* insertion in UQ406 - associated with the promoter of an α -amylase gene (Example 1, above);
- *Ds* insertion in UQ12 - associated with a putative patatin gene (Example 6, above);
- *Ds* insertion in UQ11 - associated with the Right Border of the *Agrobacterium* T-DNA in 1516E (refer to Figure 2). This was the T-DNA carrying the *Ds* that was initially transformed into tomato. In other words, the *Ds* transposed from the *Dem* locus back into the T-DNA;
- *Ds* insertion in UQ14 - associated with or closely linked to a putative sucrose synthase gene (Example 8, below); and
- *Ds* insertion in UQ13 - associated with or closely linked to a putative UDP-glucose-pyrophosphorylase gene, a gene potentially involved in starch biosynthesis.

In four of these instances, the *Ds* is associated with a gene related to carbon (C) metabolism (α -amylase, patatin, sucrose synthase and UDP-glucose-pyrophosphorylase). The lines designated UQ12 and UQ14 are also characterised by a disease mimic phenotype, implying that a patatin gene and a sucrose synthase gene (and probably other C metabolism genes) are involved in disease resistance.

- 27 -

EXAMPLE 8**Modifications of carbon metabolism**

As stated above, in four of the five lines carrying active demethylated *nos:BAR* genes, the *Ds* has
5 inserted into or near sequences homologous with carbon metabolism gene. These results
indicated that many C metabolism genes have *cis*-acting sequences which prevent methylation
and concomitant gene silencing. Demethylation sequences are inserted next to recombinant C
metabolism genes to enhance their expression and modify C metabolism in beneficial ways; such
as up-regulation of the sucrose phosphate synthase gene in sugar cane, to yield higher
10 concentrations of sugar in beneficially-modified plants.

EXAMPLE 9**Cloning of downstream genes associated with plant cell apoptosis
caused by *Ds* insertion**

15

A cDNA library is made from tomato leaf tissue showing the disease mimic (apoptosis)
phenotype caused by *Ds* insertion. This library is screened differentially with two probes, one
being cDNA from normal tissue and the other being cDNA made from leaf tissue showing the
disease mimic phenotype caused by *Ds* insertion. This procedures identifies genes specifically-
20 induced during plant cell death. These apoptosis-associated genes are then sequenced, and
compared with other genes present in the DNA databases. The proteins encoded by these genes
are expressed *in vitro* and tested for their ability to kill plant cells.

Those skilled in the art will appreciate that the invention described herein is susceptible to
25 variations and modifications other than those specifically described. It is to be understood that
the invention includes all such variations and modifications. The invention also includes all of
the steps, features, compositions and compounds referred to or indicated in this specification,
individually or collectively, and any and all combinations of any two or more of said steps or
features.

30

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- 29 -

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: THE UNIVERSITY OF QUEENSLAND

(ii) TITLE OF INVENTION: A METHOD FOR MODULATING PLANT
PHYSIOLOGICAL PROCESSES AND GENETIC
SEQUENCES USEFUL FOR SAME-II

(iii) NUMBER OF SEQUENCES: 8

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(F) ZIP: 3000

(v) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: Floppy disk

(B) COMPUTER: IBM PC compatible

(C) OPERATING SYSTEM: PC-DOS/MS-DOS

(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

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(B) FILING DATE: 25-SEP-1998

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(A) APPLICATION NO. PP3903

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(C) TELEX: AA 31787

- 30 -

(2) INFORMATION FOR SEQ ID NO:1:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 1217 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

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(2) INFORMATION FOR SEQ ID NO:2:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 1114 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

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(2) INFORMATION FOR SEQ ID NO:3:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 6263 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

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AGAACTGCTG	ATAACAAAGA	TAGCAGGGGA	ATATACAGCA	TCTTTGAAGG	AGGAACATCT	1980
GATGACCGGC	TTGATTGGGG	TCCATCTTTC	ATTTGCAGGA	ACGACACACA	ATATTCTGAT	2040
GGCACGGGGA	ATCCAGACAC	GGGTTTGGAC	TTTGAACCTG	CACCTGATAT	CGATCATCTT	2100
AATACGAGAG	TGCAGAAAGA	GTTATCAGAC	TGGATGAACT	GGCTGAAATC	TGAAATTGGA	2160

- 33 -

TTTGATGGTT	GGCGTTTCGA	TTTTGTAGG	GGATATGCAC	CTTGCATTAC	CAAATTTAT	2220
ATGGGAAACA	CGTCCCCGGA	TTTTGCTGTT	GGTGAATTGT	GGAACCTCTCT	TGCTTATGGC	2280
CAGGACGGGA	AACCGGAATA	TAACCAGGAC	AATCATAGAA	ATGAGCTAGT	TGGTTGGGTA	2340
AAAAATGCGG	GGCGGGCTGT	AACAGCTTTT	GATTTTACAA	CAAAGGGAAT	TCTTCAAGCT	2400
GCAGTTCAAG	AAGAGTTATG	GAGATTGAAG	GATCCCAATG	GAAAACCTCC	TGGGATGATC	2460
GGTGTTTTGC	CTCGAAAAGC	TGTGACTTTT	ATCGATAATC	ATGATACTGG	ATCGACACAA	2520
AATATGTGGC	CTTTCCTTC	AGACAAAGTT	ATGCAAGGAT	ATGCATACAT	TCTTACTCAT	2580
CCAGGAATCC	CATCCGTGGT	AAAAAAAATA	AATAAATTCT	TTCTACATAT	CTCATTGTTT	2640
TCTATTTTAC	AAGAAATTTA	TATTCTTTTC	CAGGGGATTT	GAGAAACTCG	GCCTGTGGGA	2700
GTTTGCTCAC	ATTGCCAGTC	TCGTAATCCA	TAAACAAACA	CTCAAACCTCT	GAGTGTGCAC	2760
ATCTAGACAC	CTCAACTCGT	TTTTCACCGT	GTTAATTGAA	CACTTCAACT	TACAAAATGA	2820
TCGTGTAGCA	CCTCCAAAAA	TTATGTGTCA	CAATTAGCCA	CGTGCAGAGAT	ACACGAAAAAT	2880
GAGTTGGAGT	AGTTAGTTGC	CAAATAAAAC	CAAGCTGAGG	TGTCTAAATG	TGCACNCTCA	2940
AAGTNGGATG	TTTACTTGGC	AGCTGAGGCC	GAGGCCATGT	TTGANTGTTA	TGCTTATAGG	3000
ATATGACACA	TTTGTTTCCG	ATTAGCTGAG	GANTTGATTA	AATCCTNGTT	TTNGTTNGCA	3060
GTTTNATNAC	CATTNCTTTG	ATNGGGGCTN	CNAGGATGGA	ATTNCAGCAC	TAANCTCTAT	3120
TAGGAAAAGG	AATAGGATTT	GTGCANCAAG	CAATGTGCAA	ATAATGGCTC	CTGATTCTGA	3180
ATCTTTATAT	ANCAATGGAT	CATCACAAAA	TCATTGTCAA	GATTGGACCA	AAACTTGATC	3240
TTGGAAATCT	TATTCCACCT	AATTATGAGG	TGGCAACTTC	TGGACAAGAC	TATGCTGTAT	3300
GGGAGCAAAA	GGCATAATCA	TATTGTACCA	CACTAAAAGG	GACCATGGCC	ACAATGGTTC	3360
TCATTAGTGT	TAATGTTATA	TGATTGAAAA	TGTAATTTAT	ATTGACATAA	TGAAGGCCAA	3420
AAATTCAAGA	AATTATAAAC	AATTCAATAG	TCCTTGCTCA	ATTCACAATT	ACATTATGAC	3480
TTCTCTATTG	CAAACTAGTT	TGGGTCCACA	TTATTGTCTC	CTAAAATTTT	ACAACATTTT	3540
TTAAGGGAAC	TTAATTAGTT	ACAGTGAACA	TATGTTGAAA	TTACCCTTTA	TCCCCTTACA	3600
ATTGATTTAA	TAAATATTTT	CCCTATCCCT	TTGGTAGTTG	GTTAGAGTTA	TAAGTAACGT	3660
AGAGATTAGT	TATAAGAGAA	TTTATGTATT	ATTATGCAGA	TGTTTAGTTA	TATCGATTTT	3720
AGTTATTTAT	ATGTTGATTA	TTTACCTTC	AATAATGCAT	ATAAAGATGG	TAAATGATTG	3780
GATTGATCGA	ATTCGAATGA	GTTTGAATAT	GAACAACTCT	TCAAATTTAA	TATAAATTTT	3840
TTTTGTCAAC	ATCTATAGCC	AAACGGCTCC	AAAACAATAA	ATAATTTACA	TTTATTGTAG	3900
TATTTTATTT	AAAATGGGAT	NTTCCTCATC	CCACTTGATC	CAGTTGAAAC	CCTAATAATA	3960
AGCCAAATCCA	ACCGTCAAAA	TTACAAATTT	TGAAAATTGC	GCTCCTCACA	GTTCTCCCCT	4020
ATTCAGATTT	GATTCATTCT	CTTCATTTTT	TGTTTTCACA	TTTTACCTCT	AAATCAACTC	4080
GAGTCCCTTT	GTTCAAATGG	GTGCTAATCA	CAGCCGTGAA	GATCTGGAGC	TTTCTGATTC	4140
CGAGTCTGAA	TCCGAATATG	GGTCCGAGTC	TCGAACAAGG	GAGGAAGAGG	AAGACGAAGA	4200

TAAC TACTCA	GATGCTAAAA	CGACGCCGTC	TTCCACTGAT	CGGAAACAGA	GCAAAACCCC	4260
GTCTTCTTTG	GATGATGTTG	AAGCAAAGCT	GAAAGCTTTA	AAGCTTAAGT	ATGGTACTCC	4320
TCATGCTAAA	ACCCCCACAG	CGAAAAACGC	TGTTAAACTT	TACCTTCATG	TTGGTG GGA	4380
CACTGCGAAT	TCCAAATGGG	TAGTTTCTGA	TAAGGTGACA	GCTTATTCGT	TTGTTAAATC	4440
GGGTAGTGAG	GATGGATCGG	ATGATGATGA	AAATGAAGAA	ACTGAGGAGA	ATGCTTGGTG	4500
GGTTTTGAAA	ATTGGGTTCG	AGGTTTCGGC	TAAGATTGAT	GAGAATTTGC	AGCTCAAGGC	4560
ATTTAAGGAG	CAGAAAAGGG	TGGATTTTGT	GGCGAATGGG	GTTTGGGCTG	TGAGATTCTT	4620
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TTATGGGTTT	GAGGCCAAATG	ATGAGAATAG	AGTTAAGGTG	TATGGTAAAG	ACTTTATGGG	4740
GTGGGCAAAT	CCAGAAGCTG	CGGATGATTC	AATGTGGGAG	GATGCTGGGG	ATAGCTTCGC	4800
GAAGAGCCCT	GCGTCTGAAA	AGAAGACACC	TTTGAGGGTT	AACCATGATT	TGAGGGAGGA	4860
GTTTGAGGAG	GCAGCTAAAG	GAGGAGCTAT	TCAGAGCTTG	GCATTAGGTG	CGTTGGATAA	4920
TAGTTTTCTT	ATAAGTGATT	CTGGAATTCA	GGTTGTGAGG	AACTATACTC	ATGGAATAAG	4980
TGGAAAAGGT	GTTTGTGTCA	ATTTTGATAA	GGAAAGGTCT	GCTGTACCTA	ATTCCACTCC	5040
AAGGAAAGCT	CTACTTCTAA	GAGCTGAGAC	TAATATGCTT	CTCATGAGTC	CAGTGACTGA	5100
TAGAAAGCCT	CACTCTCGGG	GATTACATCA	GTTTGATATC	GAGACTGGGA	AGGTTGTTAG	5160
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CTCAAGCAGT	TCCATGAGAC	AGGCTAAAAC	TGCTTTTCCA	GGCCTTG GTT	CTCCTATCAC	5520
TCATGTGGAT	GTTACCTATG	ATGGGAAGTG	GATATTGGGG	ACAACTGATA	CTTACTTGAT	5580
ATTGATATGC	ACCTTGTTTA	TCGACAAGAA	TGGAAC TACT	AAGACTGGTT	TTGCTGGTCG	5640
CATGGGAAAT	AAGATTTCCG	CTCCAAGATT	GTTAAAGCTA	AACCCTCTCG	ATTCACATAT	5700
GGCTGGAGCT	AACAAGTTCC	GCAGTGCTCA	ATTTTCATGG	GTCACCGAGA	ATGGGAAGCA	5760
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GAAGGATGGT	TCTCATGAGT	GTTACCAGAA	TCAGGT TGGG	TTGAAGAGCT	GCTATTGTTA	5880
CAAGATAGTC	CTAAGAGACG	ACTCTATTGT	AGAAAGTCGT	TTCATGCATG	ACAAGTACGC	5940
TGTTTCTGAC	TCACCTGAAG	CACCACTGGC	GGTAGCAACC	CCCATGAAAG	TCAGCTCATT	6000
CAGCATCTCT	AGCAGGCGCT	TACAAATTTG	AACAATCAT T	CTGTTCATAT	ACGCAACTTA	6060
TTAGATTTAT	CTGTAGCAGA	ATTAGTGTCT	CTCACACTAA	GTAGCTTGAA	AAACTGCACA	6120
TCTGCAAATC	ATTTCCAGTT	CAATGTATTA	CTACTTTAGT	TTAAAAACCT	TAAAAGGCAG	6180
TCTTCCAAAT	TCTAGGTATC	CTCACCTGAC	ATTATTATTG	TTGTAATAGC	TAATTGTTGC	6240

- 35 -

TTGCTCTAAA TCCCCGTTCA ATG

6263

(2) INFORMATION FOR SEQ ID NO:4:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 708 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

AAATGTAATT TATATTGACA TAATGAAGGC CAAAAATTCA AGAAATTATA AACAATTCAA	60
TAGTCCTTGC TCAATTCACA ATTACATTAT GACTTCTCTA TTGCAAACATA GTTTGGGTCC	120
ACATTATTGT CTCCTAAAAT TTTACAACAT TTCTTAAGGG AACTTAATTA GTTACAGTGA	180
ACATATGTTG AAATTACCCT TTATCCCCTT ACAATTGATT TAATAAATAT TTCCCCTATC	240
CCTTTGGTAG TTGGTTAGAG TTATAAGTAA CGTAGAGATT AGTTATAAGA GAATTTATGT	300
ATTATTATGC AGATGTTTAG TTATATCGAT TTTAGTTATT TATATGTTGA TTATTTCAAC	360
TTCAATAATG CATATAAAGA TGGTAAATGA TTGGATTGAT CGAATTCGAA TGAGTTTGAA	420
TATGAACTAA TCTTCAAATT TAATATAAAT TTTTTTGTGC AACATCTATA GCCAAACGGC	480
TCCAAAACAA TAAATAATTT ACATTTATTG TAGTATTTTA TTTAAATGG GATTCCTCA	540
TCCCACTTGT ACCAGTTGAA ACCCTAATAA TAAGCCAATC CAACCGTCAA AATTACAAAT	600
TTTGAAAATT GCGCTCCTCA CAGTTCTCCC CTATTCAGAT TTGATTCATT CTCTTCATTT	660
TTTGTTTTCA CATTTTACCT CTAAATCAAC TCGAGTCCCT TTGTTCAA	708

(2) INFORMATION FOR SEQ ID NO:5:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 684 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

AATCAAAGAG GAATTNAATT CCNCAAAATT TCATCCATAG ATTTTGNGTC	50
TCTGAAAATT AAAGTGAATT TGTAATCTGA AACCTAGAGT CCTCAACCAT	100
ATCATTGACC ATTAAGCCAT ACCCTTAAAT GTAGGGAATT TGAAGTTTAA	150
AAAACCACAC TTTGTTATTT ATTGGCCCAA ATACTCGATA ATCTTTACAT	200
TATTGAAAAT CAACATTCAA AAGGAACGAA CCTTCAATCA CACCATCAAT	250
GTCAACTTTC TTTTATTTTG GATAATCTAA GTTTTAAAT TGCAGTAAAA	300

- 36 -

TNAAATAAAA	CCCTAAACTT	CTTCTAGGTT	GAGACTTAGT	AAATATGAAT	350
TATATAAAGA	ATTCATGACA	AATGAGACAT	AAGAATAGTG	CCAGCAAATT	400
ACTTTTTTTGA	TATCTTATCT	GTGATATCGG	AATTTTAACT	ACCATAAAAT	450
TATGAATGAA	ATATCACTTA	TCTATTAGAG	AGGATTTAAT	CTCCCTTATA	500
ATGACATTGA	TAAAAGCAAG	NACAAGTGCT	CTTTATTTCT	TAATTACAAA	550
TCCTTAAATA	GATAAAAGCT	ACGAATAACA	TAATATCCTT	AAATAGATAA	600
AAGCTACGAA	TAACATAATA	GTATATTACT	CCNAATTATT	TTGATTTATT	650
TAAAATGACT	CCACTAATCC	TGATGTGGTC	TAGG		684

(2) INFORMATION FOR SEQ ID NO:6:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 662 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

GGTCTAGGCC	CTGGGTCTAG	GAAACAAAAT	AACTTATTTG	ACTCCTAAAC	50
AATAGCAACA	TACAAACCAC	TGATATTGTA	CAAGTAAAAT	TCAATAAAAT	100
TCTAGCTCTC	TCAAACACTT	TTAAAATTGT	TATTTCTGTT	TTGTCTGTGT	150
CATATTATGA	CCTACACAAC	AACAACAACA	ACGAATTTAG	TGAAACTCTA	200
CAAAGTGGAG	CCTGAAGTCG	AGAGTTTACG	CGGGCCTTAT	CACTATCTTT	250
TCGAGATAAA	AAAATTATTT	TTAAAAGATC	ATCGACTTAA	ACAAACCAAA	300
CAATAATTAA	AAAAATATGA	ATTAATAGCA	AAGCAGTGTG	GACCATATAT	350
ACAAAAATCT	ATAACAACAA	CAAGGTGCAG	AGCATTATTC	CAACTAAGAT	400
CGAAGTTGTG	ATACTGTCAT	AATAAAAATG	ACACATATTT	TGACAACATA	450
AAAAATAAAT	AACCATAAAA	TATATCATAG	AAAAATGAAT	ATATTAGAAC	500
AGCTCACTCC	AATATTAAAA	GAGAGAAAAA	AAATATTTTC	CCACCACAAT	550
GCCATAATCC	TTGAGCTTAG	CTATTTATAA	GTAAAAAATA	TGTTTTCTTG	600
GATAAATAGA	AAAAGAAATA	ATAATTAAAC	ATAACCAATC	ACTTCACAAA	650
TAAGAGTGTA	TT				662

- 37 -

(2) INFORMATION FOR SEQ ID NO:7:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 63 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

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ATTTATTTT AGGAAAAATT ATCTAAATAC ACATCTTATT TTACCATATA CTCTAAAAAT 60
TCC 63
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(2) INFORMATION FOR SEQ ID NO:8:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 63 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

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AATTATATTT AGGAAAAATT ACATAAATAC ACAACTTAAT ATATTATATT CTCTAAAATT 60
TCC 63
```

DATED this 25th day of September 1998

THE UNIVERSITY OF QUEENSLAND

By DAVIES COLLISON CAVE

Patent Attorneys for the Applicant

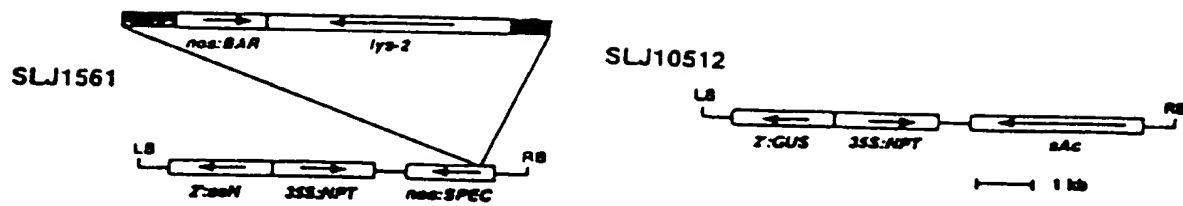


FIGURE 1

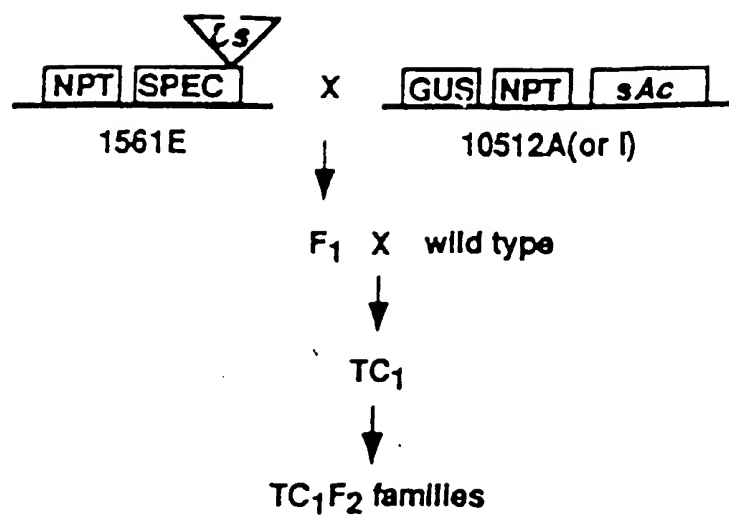


FIGURE 2

FIGURE 3 (i)

981	TTTGAAATTTATGTATATATCTGTAGCATTAGAACTATAAGAGTTGTTA	1030	Potato
40	TTTGAAATTTATGTATTTATCTATAGCATTAGAACTATAAGAGTTGTTA	89	Tomato
1031	GCTTCACTTGTCTTATTGTTGTGCTCAAAGCAACT...TCATCATACAGT	1077	
90	GCTTCACTTGGCTTACTGTTGTGCTCAAAGCAACTTCATCATACAGT	139	
1078	ATGGTTTTTATATGCTCTTCCATTATCACCGAACCTTATGATTATG.TGT	1126	
140	ATGGTTTTGATATGCTCTTCCATTATCACTGAGCCTTATGATTATGTTTT	189	
1127	ACGAGCTTATAATATTACTGATGGTGATTCAAGTATTATGATTATGTCCTC	1176	
190	ACGAGCTTATAATATCACTGATGGTGATTCAAGTATTGTGATTATGTCCTT	239	
1177	CATTAATTATTCTGTTTCATACAAGTCGTGTAATTGCTGTTTGTGATTG	1226	
240	CGTTGATTATTCTGTTTCATACAAGTCGTGTAATTGCTGTTTGTGACAG	289	
1227	TACGATAAATTGATTCAACCTTCTGCGGTGTTGGTTGAAGTTCAAGTAAA	1276	
290	TACGATAGATCGACTCAACCTTCTGAGGTATTAGTTGAAGTTCATGTAAA	339	
1277	TTAGCTTTATTTATCATAGTAGCATTGATTATTGATGCTCTGTAGCTAA	1326	
340	TTAGCTTTGTTTATCATAGTAGCATTGATTATTGATGCTCTGTAGCTAA	389	
1327	TGATAAGCCATTGAAGGGAAGCAGAAATGGTAAAGCTTTCTAAAATGAAT	1376	
390	TGATAAGCCATTGGAGGGAAGC.....AAGCTTTCT.AAATGAAT	428	
1377	CTACGAATGGATGATAAAGTTAATGAATATTGTTGATACTTCTGCAATCA	1426	
429	CTACGAATGGATGATAAAGTTCATGAATATTTTTGTTACTTCTGCAGTCA	478	
1427	GATTATGAGTTACTGAGTCTACTG.TTTTTTAAGCCTGTTTCAGATGATC	1475	
479	GATCATGAGTTATTGAGTCTATTGTTTTTTTAAGCCTGTTTCAGATGATC	528	
1476	GATCATCAACAACAACATATTCAGTGTAGTAGACATGATCGATCACTTTC	1525	
529	CATCATCAGTAACAACATACACGGTGTAGT..CCCAAATCCATCA.....	571	
1526	TAATTTTCGATTATGCACCCCTCTTTTCTCCAATTTGGTC..GTCTTCTTT	1573	
572TATGCACCTTCTTTTCTTCAATTTGGTCTTGTTTTTTTTT	610	
1574	TTTTTCATGATGTCACTGAATTATTCTCTGGTCGTCCTCCCACTTCAGGAA	1623	
611	TTTTTCATGATGTCATTGAATT.....ATTCAAGAA	640	
1624	GTC ACTTCGAG CATAATG...TGAAAACATCCACATTT.TTCAA.....	1663	
641	GTC ACTTCGAG CATAATGATTTTTTCAAAATCCACCTTTGTTCAAGCACTA	690	

UQ406
insertion

FIGURE 3 (ii)

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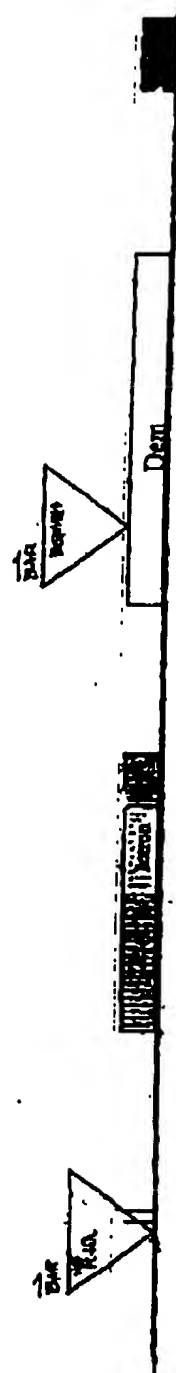
1664 .....ATCCAGC.....AGAATTTTC 1679
      ||| |||
691 CCACGTCTTTTCATCTAGCCCACAACCGTGGTGGAGGATCTAGAATTTTC 740
1680 ATCAAACGGGTTCAACATTTAC...TACATGTATACACTCTGAAGTCTG 1726
      || ||| || ||||| ||||| || ||||| || |||||
741 ATGAAA..GGATTCAAATTTACAAACATATATATACACTATACACTATG 788
1727 AATCCACTAATTCTAGATGGTGCATCTGTGCCCCCAGACTTGTGAAAGCT 1776
      ||||| ||||| ||||| ||||| ||||| ||||| |||||
789 AATCCACTAATACTAGATGGTGACCTGTGCCCCCACTCATGTGAAAGCC 838
1777 TATTCTCAATTTTTTATTTTCCAACAACCTGAATTCAGACCACACAACCTC 1826
      ||||| ||||| ||||| ||||| ||||| ||||| |||||
839 TATTCTCAATTTTTTATTTTCC.ACAACTTAAATACAGACCGCACAACTC 887
1827 CCGTGTCTTGT.....ACGGTCAGCATCTGAGTGGAGAACTCAA.... 1865
      ||||| ||||| ||||| ||||| |||||
888 CCGTGTCTTGTGTGC'CGTCGCTCAGCATGCAAGTCGAGAAAAGAAAGAC 937
1866 .....TTAAGTGACTTTAACG 1881
      ||| ||||| |||||
938 CAAAACAATGAAAACCTTTACGAAAAATCAAAAAGTTGAAGGACTTTAACG 987
1882 TCGAGTCTATAGTAAACAACCCCT.....ATATCTT 1913
      ||||| ||| ||| ||| ||| ||| |||
988 TCGAGATCTCTCGTAGAAAACCTCTTTTGTAAGGTTGCATACAATACTTT 1037
1914 TTTTCAAGCATGTTAAGATTGCGAACACACTGA..... 1946
      |||| ||| ||| ||| ||| ||| |||
1038 TTTTTCAG.ACTTTACTTATGGTATTATACTGAATATGTTATTGCTGTTA 1086
1947 .....AATTTCCAGGTCGTTAATCTTGTACC 1972
      ||||| || ||||| |||||
1087 TAGTAGTTGAGTGACGTTTGAGGGAATTTCTAGTCCGTTAATCTTGTACT 1136
1973 CAGTGTGTGTACTTTTAAAAAAAAGTCAGTTTTTTAGTCTCTAAAACA 2022
      ||||| ||||| ||||| ||||| ||||| |||||
1137 CAGTGTGTCTACTTTT...CAAAAAGTCAGTTTTTCAGTCTCTAAAACA 1183
2023 CATTTAAAT.AGAGTTTATTTG.CCATCTTTTGTTCCTCATACTAGACTT 2070
      ||||| ||||| ||||| ||||| ||||| |||||
1184 CATTTAAATAAGAGTTTCTTTGCCCATCTTTGTTCCTCATCCTAGGCTT 1233
2071 CGGAGTCAACACAACACAACAACA 2094
      ||||| ||||| ||||| |||||
1234 .GGAGTCAACACAACACAACAACA 1256

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FIGURE 4

Potato α -amylase

promoter cDNA



6/16

FIGURE 5

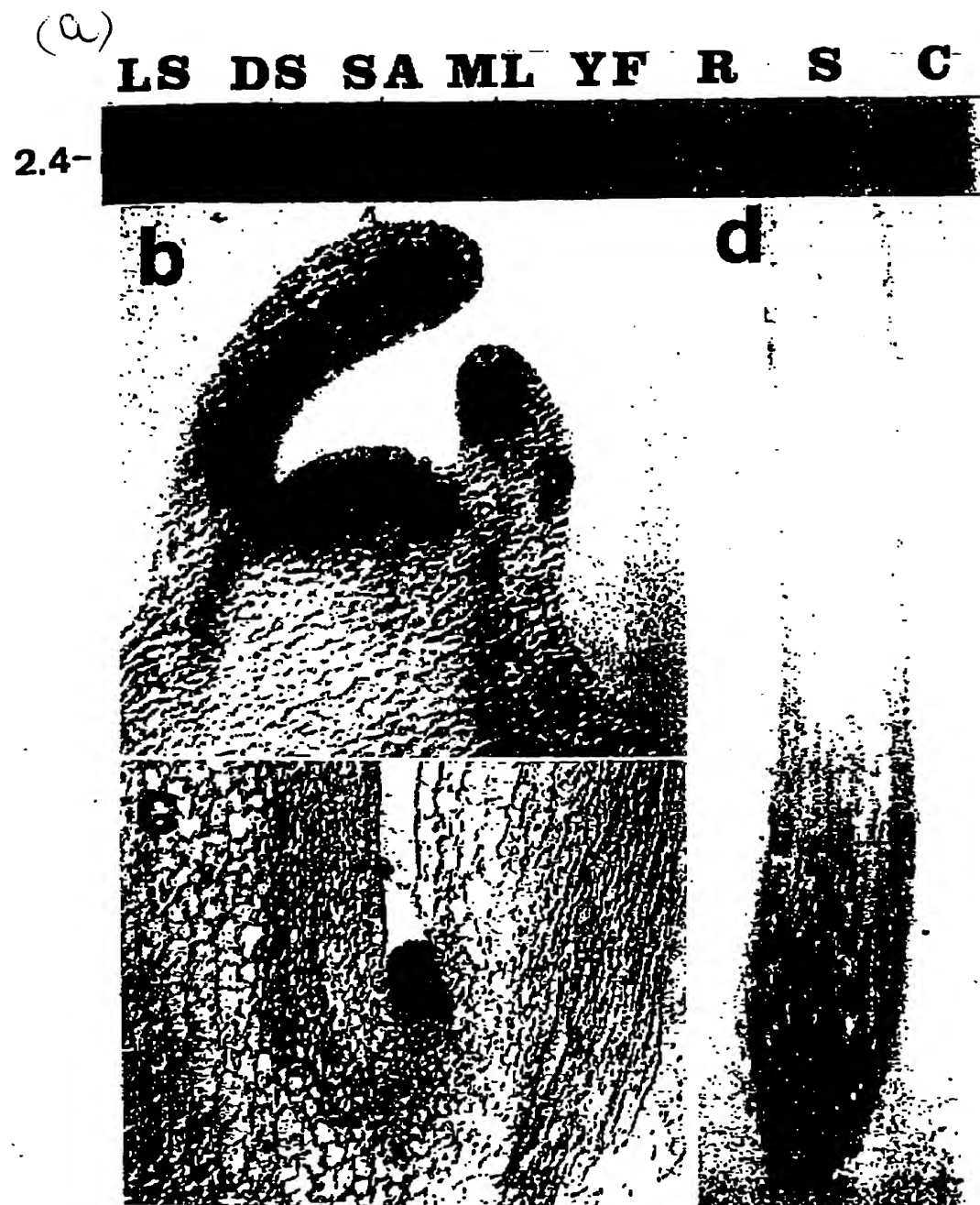
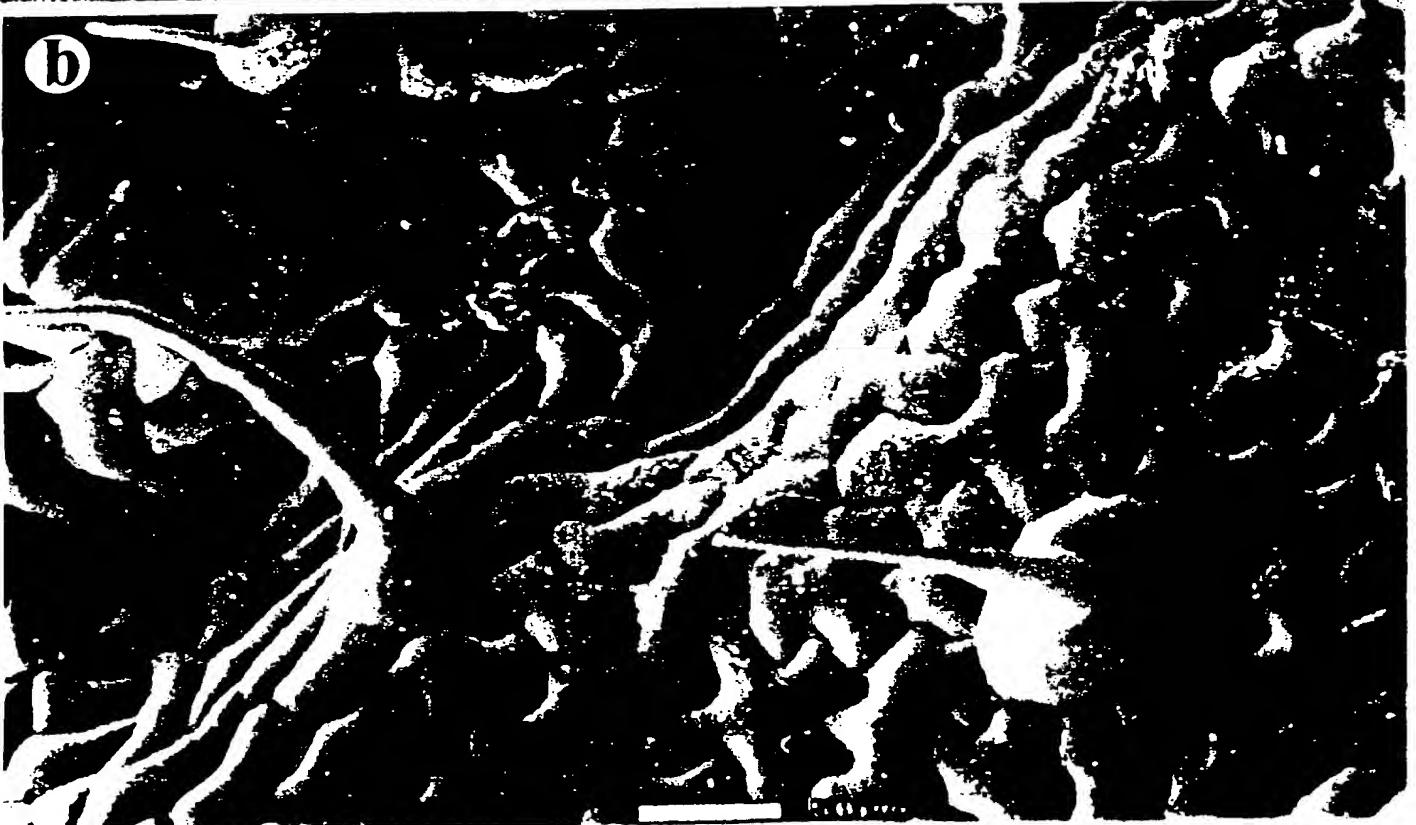
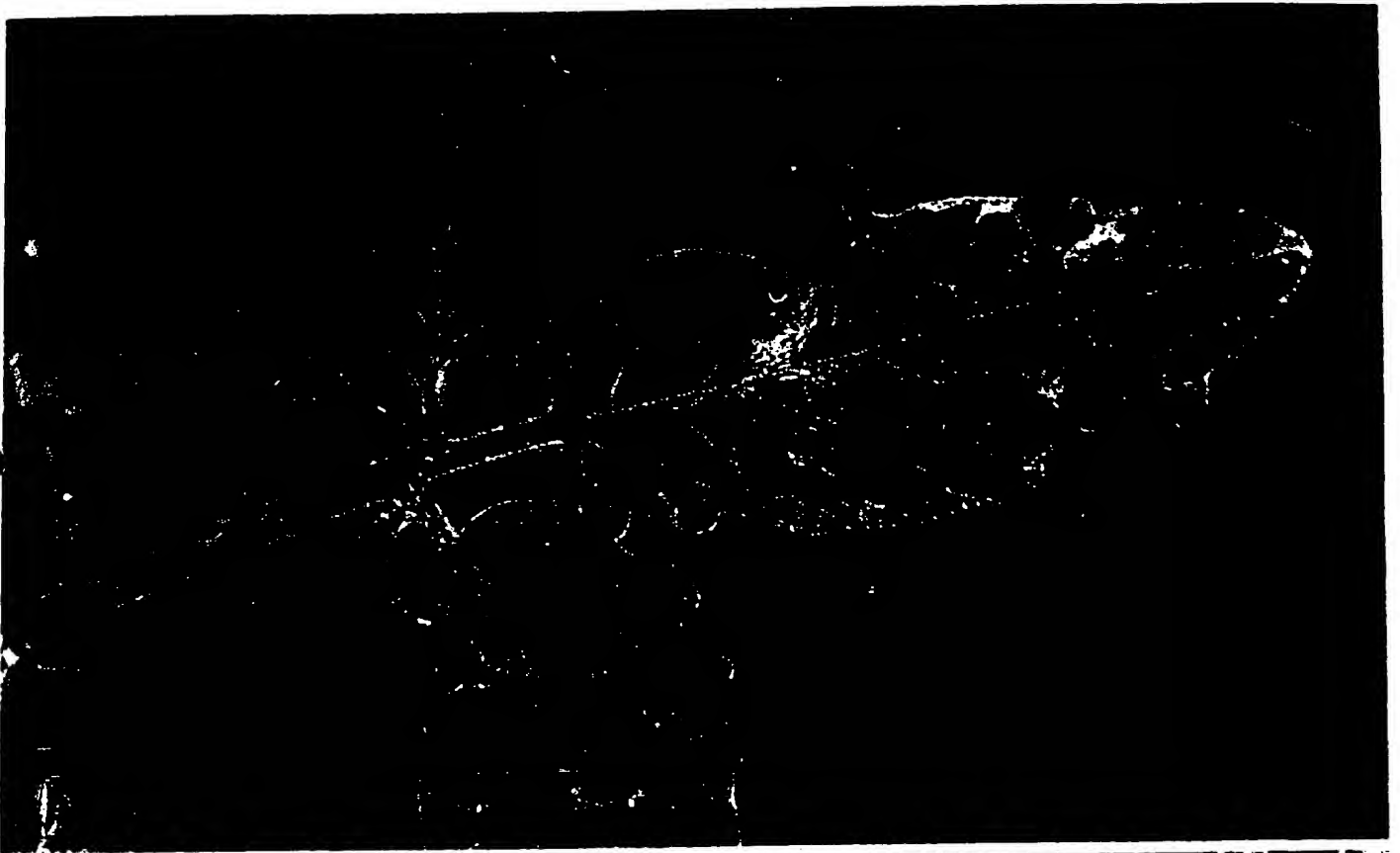


FIGURE 6



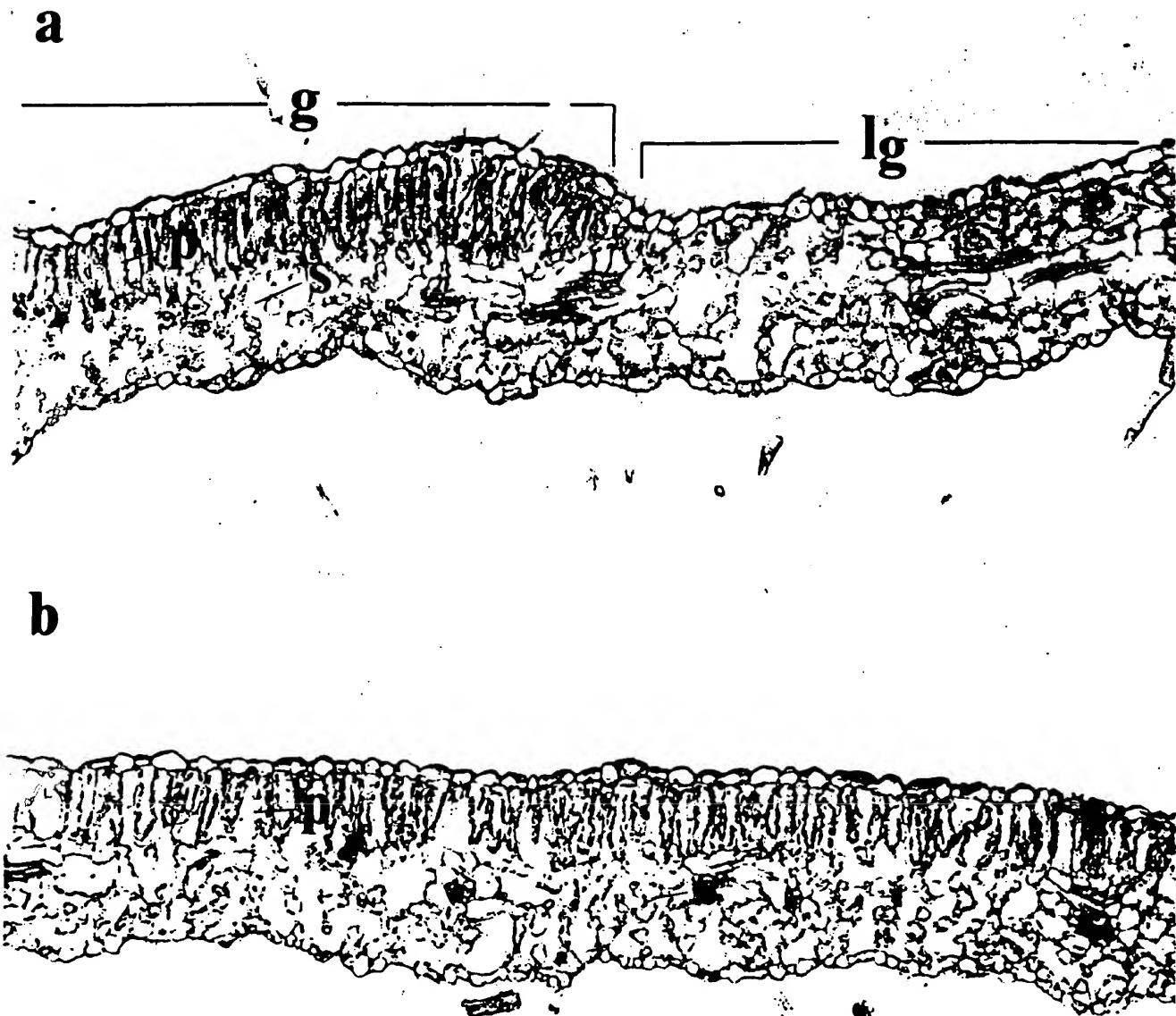
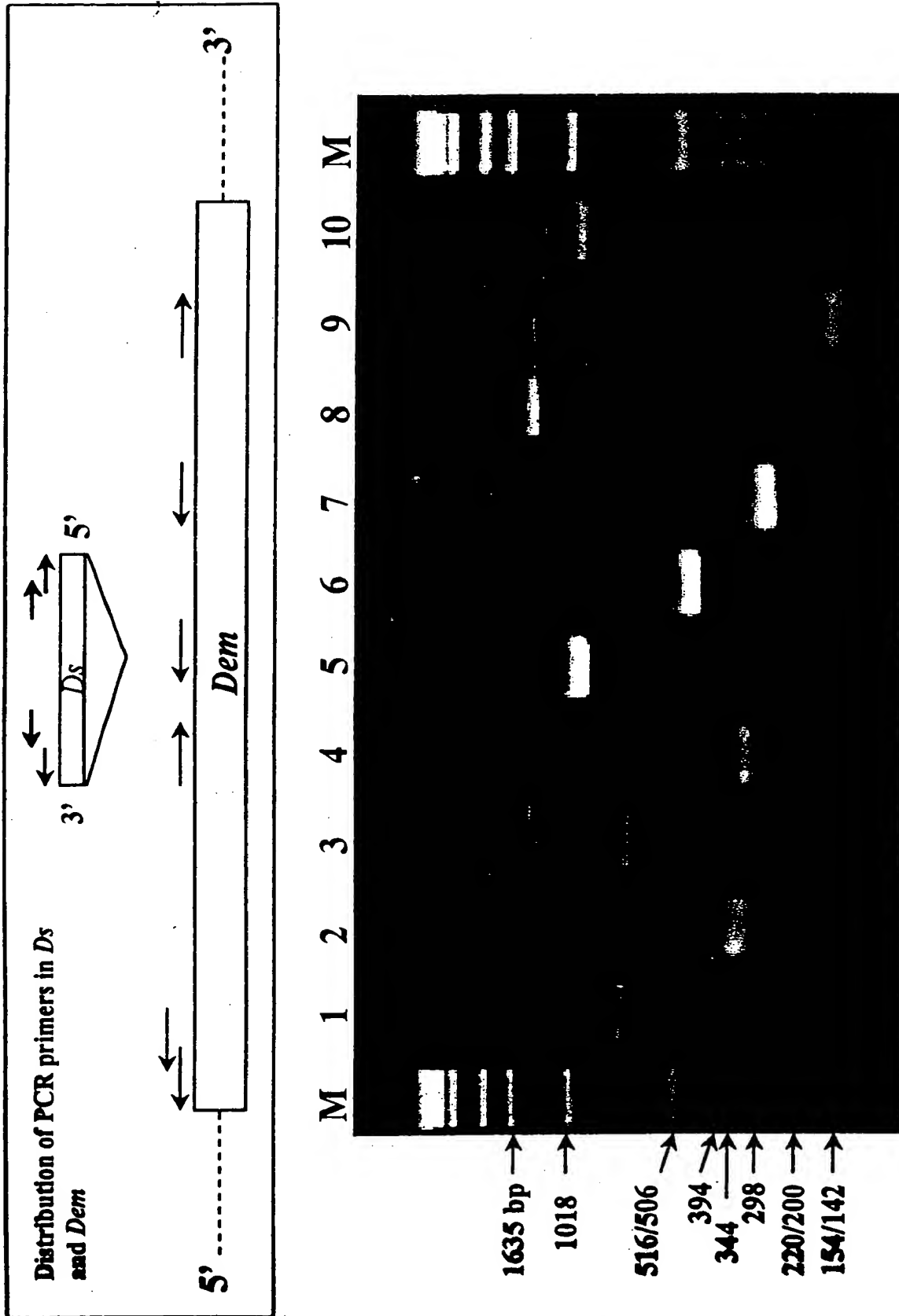


FIGURE 7

FIGURE 8



10/16

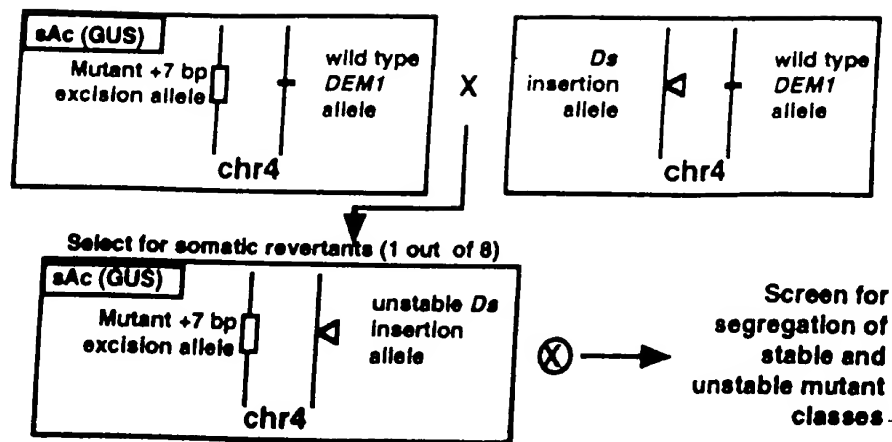


FIGURE 9

FIGURE 10 (i)

1	CGACGGCCCG	GGCTGGTAAA	TGCGGAAGCT	TGTTACAGAT	TTGAAATTTA
51	TGTATTTATC	TATAGCATT	GAAACTATAA	GAGTTGTTAG	CTTCACTTGG
101	CTTACTGTTG	TGCTCAAAGC	AACTTCATCA	TCATACAGTA	TGGTTTTGAT
151	ATGCTCTTCC	ATTATCACTG	AGCCTTATGA	TTATGTTTTA	CGAGCTTATA
201	ATATCACTGA	TGGTGATTCA	GTTTGTGAT	TATGTCCTTC	GTTGATTATT
251	CTGTTTCATA	CAAGTCGTGT	AATTTGCTGT	TTGTGACAGT	ACGATAGATC
301	GACTCAACCT	TCTGAGGTAT	TAGTTGAAGT	TCATGTAAAT	TAGCTTTGTT
351	TATCATAGTA	GCATTTGATT	ATTGATGCTC	TGTAGCTAAT	GATAAGCCAT
401	TGGAGGGAAG	CAAGCTTTCT	AAATGAATCT	ACGAATGGAT	GATAAAGTTC
451	ATGAATATTT	TTGTTACTTC	TGCAGTCAGA	TCATGAGTTA	TTGAGTCTAT
501	TGTTTTTTTT	AGCCTGTTTC	AGATGATCCA	TCATCAGTAA	CAACATACAC
551	GGTGTAGTCC	CAAATCCATC	ATATGCACCT	TCTTTTCTTC	AATTTGGTCT
601	TGTTTTTTTT	TTTTTCATGAT	GTCATTGAAT	TATTCAGAA	GTCACCTCGA
651	GCATAATGAT	TTTTCAAAAT	CCACCTTTGT	TCAAGCACTA	CCACGTCTTT
701	TCAATCTAGCC	CACAACCGTG	GTGGAGGATC	TAGAAATTTTC	ATGAAAGGAT
751	TCAAATTTTA	CAAACATATA	TATACACTAT	ACACTATGAA	TCCACTAATA
801	CTAGATGGTG	CACCTGTGCC	CCCACTCATG	TGAAAGCCTA	TTCTCAATTT
851	TTTATTTTCC	ACAACCTTAA	TACAGACCGC	ACAACCTCCG	TGTCTTGTGT
901	GCTCGTCCGT	CAGCATGCAA	GTCGAGAAAA	GAAAGACCAA	AACAATGAAA
951	ACTTTACGAA	AAATCAAAAA	GTTGAAGGAC	TTTAACGTCG	AGATCTCTCG
1001	TAGAAAACCT	CTTTTGTAAG	GTTGCATACA	ATACTTTTTT	TTTCACTTTT
1051	ACTTATGGTA	TTATAC TGAA	TATGTTATTG	CTGTTATAGT	AGTTGAGTGA
1101	CGTTTGAGGG	AATTTCTAGT	CCGTTAATCT	TGTAATCAGT	GTGTCTACTT
1151	TTCAAAAAAG	TCAGTTTTTC	AGTCTCTAAA	ACACATTTAA	ATAAGAGTTT
1201	CTTTGCCCAT	CTTTTGTTCC	TCATCCTAGG	CTTGGAGTCA	ACACAACACA
1251	ACAACAATGA	ATTTCATTT	TTCTGTTTCT	TTACTTCTCT	CTTTATCTCT
1301	TCCTATGTTT	GCCTCTTCGA	CGGTGTTATT	TCAGGTATCC	ATCTCCAAAG
1351	AACCTTATTT	TTCTCTTAAC	TTTTCTTATG	TATATGTATC	TCTATGTTTA
1401	TGTAGTACTT	GCTCAAGTAT	ATAAAGAAAA	GTTAGTTTCT	CTAGAATCTT
1451	TGAATTCATT	TGTTAGGGGT	TCAATTGGGA	TTCGAGTAAT	AAGCAAGGCG
1501	GATGGTACAA	CTCTCTCATC	AACCTAGTTC	CGGACTTGGC	TAAAGCTGGA
1551	GTTACTCATG	TTTGGTTGCC	ACCATCATCT	CACCTCCGTT	CTCCTCAAGG
1601	TAATTTTCGG	AGTGATTGTG	ACCTAGTAAT	CCAATGAAGT	CAAAATAACC
1651	ACGGAAGATT	AGAGTCTAAA	TTTTAATGAA	AATAGTTCAG	ACAAGTTAAT
1701	GACCAACTTA	TATATTAGTT	CAATCCATAA	AATTTGATGT	AGTAGTTACA
1751	AAATGGAATT	GCTTGAAGGC	TTATGCCATG	TTTTATGCCA	GGTTATATGC
1801	CAGGAAGGTT	GTATGACTAG	GATGCTTCCA	AGTTTGGAAA	TCAGCAACAA
1851	CTGAAAACCT	TTATTAAGGC	TTTAACATGA	CCACGGGATC	AAATCGGTTG
1901	CTGATATAGT	GATAAATCAT	AGAAGTCTCT	GATGACCGGC	TTGATTGGGG
1951	ATATACAGCA	TC'TTTGAAGG	ACGACACACA	ATATTCTGAT	GGCACGGGGA
2001	TCCATCTTTC	ATTTGCAGGA	TTTGAACCTG	CACCTGATAT	CGATCATCTT
2051	ATCCAGACAC	GGGTTTGGAC	GTTATCAGAC	TGGATGAAC	GGCTGAAATC
2101	AATACGAGAG	TGCAGAAAGA	GGCGTTTCGA	TTTTGTTAGG	GGATATGCAC
2151	TGAAATTTGA	TTTGATGGTT	ATGGGAAACA	CGTCCCCGGA	TTTTGCTGTT
2201	CTTGCAATTAC	CAAAATTTAT	TGCTTATGGC	CAGGACGGGA	AACCGGAATA
2251	GGTGAATTGT	GGAAGTCTCT	ATGAGCTAGT	TGGTTGGGTA	AAAAATGCGG
2301	TAACCAGGAC	AATCATAGAA	GATTTTACAA	CAAAGGGAAT	TCTTCAAGCT
2351	GGCGGGCTGT	AACAGCTTTT	GAGATTGAAG	GATCCCAATG	GAAAACCTCC
2401	GCAGTTCAAG	AAGAGTTATG	CTCGAAAAGC	TGTGACTTTT	ATCGATAATC
2451	TGGGATGATC	GGTGTPTTGC	AATATGTGGC	CTTTCCCTTC	AGACAAAGTT
2501	ATGATACTGG	ATCGACACAA	TCTTACTCAT	CCAGGAATCC	CATCCGTGGT
2551	ATGCAAGGAT	ATGCATACAT	TTCTACATAT	CTCATTGTTT	TCTATTTTAC
2601	AAAAAAATA	AATAAATCT	CAGGGGATTT	GAGAAACTCG	GCCTGTGGGA
2651	AAGAAATTTA	TATTC'TTTT	TCGTAATCCA	TAAACAAACA	CTCAAACCTC
2701	GTTTGCTCAC	ATTGCCAGTC	CTCAACTCGT	TTTTCAACCG	GTTAATTGAA
2751	GAGTGTGCAC	ATCTAGACAC	TCTGTAGTCA	CCTCCAAAAA	TTATGTGTCA
2801	CACTTCAACT	TACAAAATGA	ACACGAAAAT	GAGTTGGAGT	AGTTAGTTGC
2851	CAATTAGCCA	CGTGCGAGAT	TGTCTAAATG	TGCACNCTCA	AAGTNGGATG
2901	CAAAATAAAC	CAAGCTGAGG	GAGGCCATGT	TTGANTGTTA	TGCTTATAGG
2951	TTTACTTGGC	AGCTGAGGCC	ATTAGCTGAG	GANTTGATTA	AATCCTNGTT
3001	ATATGACACA	TTTGTTTCCG	CATTNCTTTG	ATNGGGGCTN	CNAGGATGGA
3051	TTNGTTNGCA	GTTTNATNAC	TAGGAAAAGG	AATAGGATTT	GTGCANCAAG
3101	ATTNCAGCAC	TAANCTCTAT			

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FIGURE 10 (ii)

3151 CAATGTGCAA ATAATGGCTC CTGATTCTGA ATCTTTATAT ANCAATGGAT
 3201 CATCACAAAA TCATTGTCAA GATTGGACCA AAACCTTGATC TTGGAAATCT
 3251 TATTCCACCT AATTATGAGG TGGCAACTTC TGGACAAGAC TATGCTGTAT
 3301 GGGAGCAAAA GGCATAATCA TATTGTACCA CACTAAAAGG GACCATGGCC
 3351 ACAATGGTTC TCATTAGTGT TAATGTTATA TGATTGAAAA TGTAATTTAT
 3401 ATTGACATAA TGAAGGCCAA AAATCAAGA AATTATAAAC AATTCAATAG
 3451 TCCTTGCTCA ATTCACAATT ACATTATGAC TTCTCTATTG CAAACTAGTT
 3501 TGGGTCCACA TTATTGTCTC CTAAAATTTT ACAACATTTC TTAAGGGAAC
 3551 TTAATTAGTT ACAGTGAACA TATGTTGAAA TTACCCTTTA TCCCCTTACA
 3601 ATTGATTTAA TAAATATTTC CCCTATCCCT TTGGTAGTTG GTTAGAGTTA
 3651 TAAGTAACGT AGAGATTAGT TATAAGAGAA TTTATGTATT ATTATGCAGA
 3701 TGTTTAGTTA TATCGATTTT AGTTATTTAT ATGTTGATTA TTTCACCTTC
 3751 AATAATGCAT ATAAAGATGG TAAATGATTG GATTGATCGA ATTCTGAATGA
 3801 GTTTGAATAT GAACATAACT TCAAATTTAA TATAAATTTT TTTTGTCAAC
 3851 ATCTATAGCC AAACGGCTCC AAAACAATAA ATAATTTACA TTTATGTAG
 3901 TATTTTATTT AAAATGGGAT NTTCCTCATC CCACTTGATC CAGTTGAAAC
 3951 CCTAATAATA AGCCAATCCA ACCGTCAAAA TTACAAATTT TGAAAATTGC
 4001 GCTCCTCACA GTTCTCCCTT ATTCTAGATT GATTCAATCT CTTCATTTT
 4051 TGTTTTTACA TTTTACCTCT AAATCAACAA AATTCCTTTT GTTCAAATGG Dem ATG
 4101 GTGCTAATCA CAGCCGTGAA GATCTGGAGC TTCTGTATTC CGAGCTTGAA
 4151 TCCGAATATG GGTCCGAGTC TCGAACAAGG GAGGAAGAGG AAGACGAAGA
 4201 TAACTACTCA GATGCTAAAA CGACGCCGTC TTCCACTGAT CGGAAACAGA
 4251 GCAAAACCCC GTCTTCTTTG GATGATGTTG AAGCAAGCT GAAAGCTTTA
 4301 AAGCTTAAGT ATGGTACTCC TCATGCTAAA ACCCCACAG CGAAAAACGG
 4351 TGTTAAACTT TACCTTCATG TTGGTGGGAA CACTGCGAAT TCCAAATGGG
 4401 TAGTTTCTGA TAAGGTGACA GCTTATTCGT TTGTTAAATC GGTAGTGAG
 4451 GATGGATCGG ATGATGATGA AAATGAAGAA ACTGAGGAGA ATGCTTGGTG
 4501 GGTTTTGAAA ATTGGGTGGA AGGTTGGGGC TAAGATTGAT GAGAATTGTC
 4551 AGCTCAAGGC ATTAAAGGAG CAGAAAAGGG TGGATTTTGT GCGAATGGG
 4601 GTTTGGGCTG TGAGATTCTT TGGGGAGGAA GAGTATAAGG CGTTCATTGA
 4651 CTATATATCAG AGCTGTTTGT TTGAGAATAC TTATGGGTTT GAGGCAAATG
 4701 ATGAGAATAG AGTTAAGGTG TATGGTAAAG ACTTTATGGG GTGGGCAAAT
 4751 CCAGAAGCTG CGGATGATTC AATGTTGGAG GATGCTGGGG ATAGCTTCGG
 4801 GAAGAGCCCT GGTCTGAAA AGAAGACACC TTTGAGGGTT AACCATGATT
 4851 TGAGGGAGGA GTTTGAGGAG GCAGCTAAG GAGGAGCTAT TCAGAGCTTG
 4901 GCATTAGGTG CGTTGGATAA TAGTTTCTT ATAAGTGATT CTGGAATTCA
 4951 GGTGTGAGG AACTATACTC ATGGAATAAG TGGAAAAGGT GTTTGTGTCA
 5001 ATTTTGATTA GGAAGGTCT GCTGTACCTA ATTCCACTCC AAGGAAAGCT
 5051 CTACTTCTAA GAGCTGAGAC TAATATGCTT CTCATGAGTC CAGTGACTGA
 5101 TAGAAAGCCT CACTCTCGGG GATTACATCA GTTTGATATC GAGACTGGGA
 5151 AGGTGTTAG CGAGTGAAG TTTGAGAAAG ATGGAATGA TATCAGGATG
 5201 AGGGATATCA CTAATGATAG CAAAGGAGCT CAGATGGATC CTTCGGGGTC
 5251 TACTTCTTA GGGCTAGATG ATAACAGATT GTGTAGGTGG GATATGCGTG
 5301 ATCGGCATGG GATGGTCCAG AATCTAGTTG ATGAAAGTAC TCCTGTGCTG
 5351 AATTGAGCTC AAGGACATCA ATTTTCGAGG GGAACCTAAT TTCAGTGCTT
 5401 TGCTACTACT GGTGATGGAT CAATTGTTGT TGGTCACTT GATGGCAAGA
 5451 TTAGATTGTA CTCAAGCAGT TCCATGAGAC AGCCTAAAAC TGCTTTTCCA
 5501 GGCCTTGGTT CTCTATCAC TCATGTGGAT GTTACCTATG ATGGGAAGTG
 5551 GATATTGGGG ACAACTGATA CTACTTGAT ATTGATATGC ACCTTGTTA
 5601 TCGACAAGAA TGGAACTACT AAGACTGGTT TTGCTGGTGG CATGGGAAAT
 5651 AAGATTTCCG CTCCAAGATT GTTAAAGCTA AACCTCTCG ATTACATAT
 5701 GGCTGGAGCT AACAAAGTCC GCAGTGCTCA ATTTTCATGG GTCAAGGAGA
 5751 ATGGGAAGCA AGAGCGCCAC CTGTTGCTA CTGTTGGGAA GTTAGAGTG
 5801 ATCTGGAATT TCAACAGGT GAAGGATGGT TCTCATGAGT GTTACCAGAA
 5851 TCAGGTTGGG TGAAGAGCT GCTATTGTTA CAAGATAGTC CTAAGAGAGC
 5901 ACTCTATTGT AGAAGTCTGT TTCATGCATG ACAAGTACGC TGTTTCTGAC
 5951 TCACCTGAAG CACCACTGGC GGTAGCAACC CCAATGAAG TCAGCTCATT
 6001 CAGCATCTCT AGCAGGGGCT TACAAATTTG AACAAATCAT CTGTTTCATAT
 6051 ACGCAACTTA TTAGATTAT CTGTAGCAGA ATTAGGTCT CTACACTAA

FIGURE 10 (iii)

6101 GTAGCTTGAA AAACTGCACA TCTGCAAATC ATTTCCAGTT CAATGTATTA
6151 CTACTTTAGT TTAAAAACCT TAAAAGGCAG TCTTCCAAAT TCTAGGTATC
6201 CTCACCTGAC ATTATTATTG TTGTAATAGC TAATTGTTGC TTGCTCTAAA
6251 TCCCCGTTCA ATG

14/16



FIGURE 11

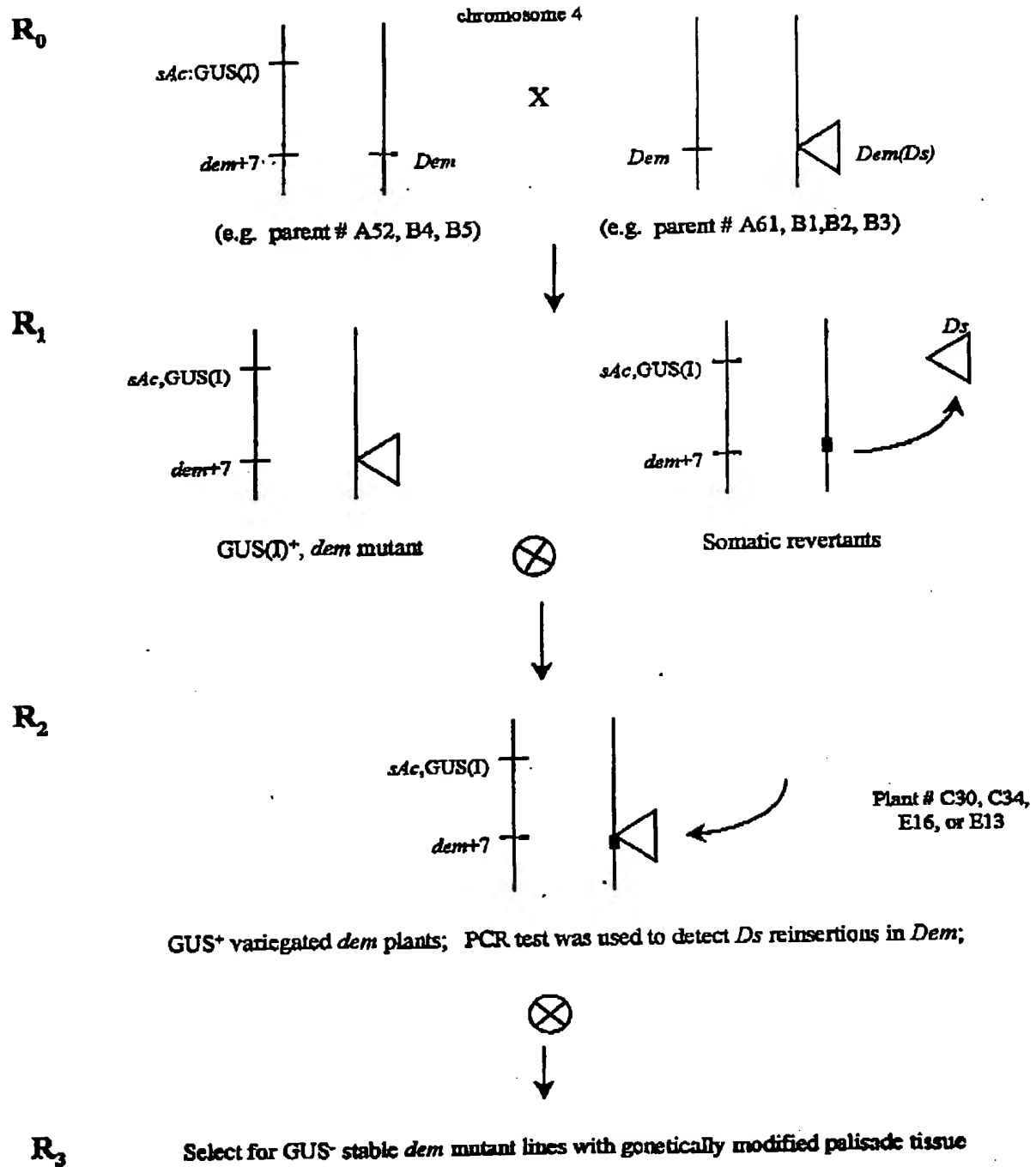


FIGURE 12



FIGURE 13